

THE TRANSLATED CONCEPTUAL SURVEY OF PHYSICS / STABLIZATION
OF THE FOCAL PLANE IN TWO PHOTON EXCITATION
FLUORESCENCE MICROSCOPY

by
Asma wada

A thesis submitted to the Faculty and the Board of Trustees of the Colorado School of Mines
in partial fulfillment of the requirements for the degree of Master of Science (Applied Physics).

Golden, Colorado

Date _____

Signed: _____

Asma Wada

Signed: _____

Dr. Patrick Kohl

Thesis Advisor

Golden, Colorado

Date _____

Signed: _____

Dr. Tom Furtak

Professor and Head

Department of Engineering Physics

ABSTRACT

As a reflection of my career to be an effective college physics teacher, my thesis is in two parts. The first is in education research, the focus of this part is to have a tool to evaluate pedagogies I have learned at the school and plan to apply in my classrooms back home. Consequently, this resulted in the development of the translated conceptual survey of physics (**TCSP**).

(TCSP) was designed by combining some questions from the Force Conceptual Inventory (**FCI**), and the Conceptual Survey of Electricity and Magnetism (**CSEM**) to assess student's understanding of basic concepts of Newtonian mechanics and electricity and magnetism in introductory physics. The idea of developing this questionnaire is to use it in classrooms back home as a part of a long term objective to implement what has been realized in the area of education research to improve the quality of teaching physics there. The survey was initially written in English, validated with interviews with native English speakers, translated into Arabic, and then validated via an interview with a native Arabic speaker. We then administered the survey to two different English-speaking intro physics courses and analyzed the results for consistency.

The objective of the second part in my thesis is to expand my knowledge in an area of physics that I have interest in, and getting involved in a scientific research to develop skills I need as a teacher. My research is in optical physics; in particular, I am working on one of the challenges in implementing two photon excitation fluorescence (**TPEF**) microscopy in imaging living systems. (**TPEF**) microscopy has been shown to be an invaluable tool for investigating biological structure and function in living organisms. The utility of (**TPEF**) imaging for this application arises from several important factors including it's ability to image deep within tissue, and to do so without harming the organism. Both of these advantages arise from the fact that (**TPEF**) imaging is done with excitation wavelengths in the near infrared (**NIR**). The (**NIR**) wavelength regime, 750-1100nm, penetrates deep ($>100 \mu\text{m}$) into tissue, and has been used to image to depths of up to 1 mm. Further, the longer excitation wavelengths are less absorbing than the traditional ultraviolet wavelengths used in confocal microscopy, and are consequently less damaging. As a result (**TPEF**) is presently the preferred tool for visualizing dynamics by

biologists. One important aspect of imaging living systems, however, is that they move! This adds to the challenge of trying to study some particular biological function(s). This thesis begins to address this issue by combining a simple micro controller circuit that can be linked to a remote focusing scheme that will make it possible to lock a focal plane to a specific depth inside a living, moving specimen.

TABLE OF CONTENTS

ABSTRACT.....	iii
LIST OF FIGURES AND TABLES.....	viii
AKNOWLEDGMENTS.....	ix
DEDICATION.....	x
CHAPTER 1 INTRODUCTION.....	1
1.1A Description and a Brief History of PER in the US.....	1
1.2 Research Areas in PER.....	2
1.2.1 Conceptual Understanding.....	2
1.2.2 Epistemology.....	3
1.2.3 Problem Solving.....	3
1.2.4 Attitudes.....	4
1.2.5 Social Aspects.....	4
1.2.6 Technology.....	5
1.2.7 Evaluation of Specific Instructional Intervention.....	5
1.2.8 Instructional Materials.....	6
1.3 The Focus of this Work.....	6
CHAPTER 2 Methods.....	7
2.1 Questions Selection.....	7
2.2 Translation Process.....	7
2.3 Test Validity.....	9
CHAPTER 3 Data Analysis.....	11

3.1 Data Analysis for PH100 Students.....	11
3.2 Data Analysis for PH200 Students.....	12
3.3 Statistical techniques used.....	13
CHAPTER 4 CONCLUSION AND POSSIBLE ABLICATIONS BACK HOME.....	15
4.1 Review of Test Data.....	15
4.2 Applications of the Test Back Home.....	15
CHAPTER 5 INTRODUCTION.....	17
5.1 WHAT IS TWO PHOTON FLUORESCENCE (TPEF) MICROSCOPY.....	17
5.2 Applications.....	18
5.2.1 Developmental Biology.....	18
5.2.2 Calcium Imaging.....	18
5.2.3 <i>In vivo</i> Imaging.....	19
5.3 The Focus of this Work.....	20
CHAPTER 6 REMOTE FOCUSING TECHNIQUE.....	22
6.1 Refocusing Techniques in Microscopy.....	22
6.2 Remote Focusing technique.....	23
6.3 Measuring the Point Spread Function.....	24
CHAPTER 7 INTRODUCTION TO MICROCONTROLLERS.....	27
7.1 Arduino Uno Board.....	27
7.2 Arduino Capability.....	30
7.3 Programming the Uno Board.....	30
CHAPTER 8 CONSTRUCTING AND PERFORMANCE OF A PIEZO CONTROLI- NG CIRCUIT.....	32
8.1 Constructing the Circuit.....	32

8.2 Circuit Performance.....	36
8.3 Conclusion and Applications to the Field of Microscopy.....	38
8.4 Review of Achieved work.....	39
REFERENCES CITED	40
APPENDIX A.....	45

LIST OF FIGURES AND TABLES

Figure 6.1 Remote focusing microscope (Image taken from (E. Botcherby <i>et al.</i> 2008 b)).....	23
Figure 6.2 Traditional refocusing method (Image taken from (E. Botcherby <i>et al.</i> 2008 a)).....	25
Figure 6.3 PSFs measured for system refocuses via: (a) Traditional technique (b) Remote focusing technique (Image taken from (E. Botcherby <i>et al.</i> 2008 a)).....	26
Figure 7.1 The Arduino Uno board.....	28
Figure 7.2 Pulse width modulation: (Image taken from (www.arduino.cc/en/Tutorial/PWM)).....	29
Figure 8.1 Emitter amplifier and low pass filter circuit.....	32
Figure 8.2 $V_{out} = 10V$ when $V_{in} = 0$	33
Figure 8.3 $V_{out} = 0$ when $V_{in} = 5V$	33
Figure 8.4 (a) The magnitude of the transfer function, (b) The phase of the transfer function (image taken from Allan r. Hambley 2008).....	34
Figure 8.5 Piezo controlling circuit.....	35
Figure 8.6 V_{out} versus time as obtained from the oscilloscope.....	38
Figure 8.7 The picture illustrates V_{out} versus time: (a) with out the filter. (b) With the filter.....	38
Figure 8.8 Schematic diagram of the remote focusing microscope linked to the piezo controlling circuit.....	39
Table 2.1 Newtonian concepts in the test.....	8
Table 2.2 Electricity and magnetism concepts in the survey.....	8
Table 3.1 The mean and standard deviation for PH100 and PH200 students.....	13

AKNOWLEDGMENT

I would like to thank Dr. Jeff Squier for his unlimited motivation, and Dr. Patrick Kohl and Dr. Tom Furtak for their continued support through my program.

For my dear family

CHAPTER 1

INTRODUCTION TO PHYSICS EDUCATION RESEARCH

Significant learning gains, developing critical thinking skills, the ability to apply what is taught in classrooms to the real world, and more outcomes that are resulting from the implementation of research-based instruction have led to the recent recognition of Physics Education Research (PER) and its importance effect to teaching physics. This chapter summarizes what PER is and what areas of research dose it cover showing its benefits to teaching physics in college level.

1.1 A Description and A Brief History of PER in the US:

PER investigates what happens as students try to learn and develop the concepts of physics and what affect this, via qualitative and quantitative research. Repeated research with many students at different places may show repeated patterns of responses that can lead to theories that help to improve physics instruction (Robert Beichner 2009). Lillian McDermott at the University of Washington is considered as the “founder” of college-level PER in the United States, and the Washington group is one of the largest PER groups in the world. Other current US PER groups include: North Carolina State University, University of Maine, University of Colorado, University of Maryland, and the Ohio State University (Robert Beichner). Along with many individuals who had a recognized influence on the field. A “family tree” was produced by Michael Wittmann showing who influenced who is available at (<http://www.umit.maine.edu/~Wittmann/FamilyTree.jpg>).

In an effort to recognize PER as a scientific research field, and to develop the studies relevant to it, there was a series of meetings that resulted in the PER conference (PERC) that is held every summer since 1997, the biennial conference series organized by the University of Maine since 2005, and regular PER sessions at the meetings of the American Physics Society APS. An outcome of one of the first of these meetings (Fall 1994, North Carolina State University) was the 1995 white paper submitted by representatives to the physics education researchers. The paper recommended that the National Science Foundation NSF Physics Division establishes PER as a subfield of physics and support the field on regular basis (Robert B. *et al.* 1995).

An important event in the history of PER was the publication of the force concept inventory (FCI) (D. Hestenes. *et al.* 1992). The (FCI) is an instrument designed to assess students understanding of the basic concepts in Newtonian mechanics. The instrument was based on the work of Halloun (I. Halloun & D. Hestenes 1985) and it has been developed and revised (I. Halloun *et al.* 1995). The (FCI) played a significant role in the development of (PER) as it revealed that students in interactive classes (often classes that implement research based instruction) performed better than student in traditional classes (R. Hake 1998). This led to the recognition of one key PER methods: evaluating the effectiveness of instruction via pre-post testing using validated tests.

As the field of PER became more recognized, there has been different resources of publications for learning about the field. One of the key resources for that is the American Journal of Physics (Lillian M. & Edward R.). The American Association of Physics Teachers AAPT and the American Physics Society with their publication of Physical Review Special Topics-Physics Education Research since 2005 is another source for PER studies. In addition to other journals that occasionally include articles relevant to PER (Robert B. 2009).

1.2 Research Areas in PER:

In an effort to improve teaching and learning physics, PER has branched into different areas of studies. Below is a description of some of these areas:

1.2.1 Conceptual Understanding:

Researchers in this area are looking at how and what students learn of the concepts of physics. An early example of this type of research is the investigation of students understanding of the concept of velocity in one dimension at the University of Washington (D. Trowbridge & L. McDermott 1980). In this study students in introductory college physics courses were interviewed and asked specific questions to asses their ability to apply the concept of velocity to interpret simple motions of real objects. A Similar study on students understanding of the concept of acceleration has been conducted by the same authors. In both studies students' difficulties in applying the concepts and discriminating between different concepts were identified. Implications for instruction-to deal with those difficulties- have been discussed.

Part of this type of research is the invention of assessment instruments in topics of physics where students' difficulties have been identified. Examples of these tests are: the (FCI) mentioned earlier, the Force and Motion Conceptual Evaluation (FMCE) (R. Thornton & D. Sokoloff 1998), the Test of Understanding Graphs in Kinematics (TUG-K) (R.J. Beichner 1994), and more. The main purpose of these tests is to evaluate the effectiveness of instruction.

Other sub-areas under conceptual understanding research are: the work of DiSessa in analyzing students reasoning in introductory physics (DiSessa 1988). DiSessa found out that many students come up with simple statements (which DiSessa called *phenomenological primitives*) to describe the way they think of physics conceptions and how things work in the world around them. For example students refer to "force as a mover", and this is often misdiagnosed as "misconception". DiSessa's work has a great impact on research trying to understand students thinking. Also, there have been researchers who investigate how students' ideas are changed as they learn (D. E. Brown & David Hammer 2008).

1.2.2 Epistemology:

Researchers in this field are working on theories of the cognition involved in learning physics which represents the theoretical basis of PER. Redish, for example, proposed a theoretical framework explaining what is seen in classroom and comparing different theoretical methods (Edward F. Redish 2004). He gave an analysis of student's cognition and its interaction with the environment, sketched some models for students thinking and learning in physics, and he gave examples of how theoretical studies can have an impact on instruction and research. In another study, Hammer explores how students epistemological beliefs (beliefs about knowledge and learning) can affect instruction (David H. 1995). His study was in the context of a discussion about velocity in high school physics class.

1.2.3 Problem Solving:

Instructors would like their students to be able to apply what they learn from lectures and text books to solve physics problems. However this is a difficult task for most students in a class. Studying how students solve and learn to solve problems can be helpful to increase number of students who are competent problem solvers. Researchers in this field investigate the cognitive process involved in problem solving, study the effect of curricular materials on student's learning,

develop computer programs to help students learn to solve problems, and study the differences between novices and experts as they solve problems. A review on the topic is presented in the resource letter provided by (Leonardo H. *et al.* 2004).

Examples of studies done under this field are: Comparison between representations used by experts and novices when solving physics problems (Michelene T. H. *et al.* 1981). Authors in this study showed that expert's representations are based on abstract physics principles, while novices base their representations on the problem's text. Another study was at the University of Minnesota (Patericia H. *et al.* 1992) aiming to teach students an effective problem-solving strategy through forming cooperative groups with specific structure and giving them context-rich problems.

1.2.4 Attitudes:

Studies on students' beliefs and attitudes toward science (House, J.D. 1995) indicate that those attitudes are better in predicting college science student's performance than the amount of previous science and math they have completed. A number of surveys have been designed to measure student beliefs about physics and learning physics. The Colorado Learning Attitudes about Science Survey (CLASS) (W. K. Adams *et al.* 2006) is one of these surveys. The (CLASS) is designed based on existing work to investigate how students beliefs impact and are impacted by their learning experience, and to distinguish between beliefs of experts and novices. Students are asked to respond on a 5-point scale (agree- disagree) to 42 clear statements like:

“I study physics to learn knowledge that will be useful in my life outside of school”. The survey results revealed that students' beliefs toward physics tend to decline after traditional instruction; however instruction aimed at addressing students' beliefs about physics has a positive measurable effect. It also shows that there are large gender differences in beliefs. Another example of those instruments is the Maryland Physics Expectations Survey (MPEX) (E. F. Radish *et al.* 1998). The survey has been developed to see how students see physics and how they think they work in an introductory calculus-based physics course.

1.2.5 Social Aspects:

Non-academic aspects such as: gender, race, learning environment, student interactions can easily impact students performance. In PER there are studies on the effect of these aspects on

learning and teaching physics. For example, the study was done by Williams (Karen Williams 2001) documenting women's fear about communications in physics classes even though they learned about force concepts as much as men did during the course. Also, consider studies about studio classrooms such that carried out by (Cummings *et al.* 1999) at Rensselaer. There the authors investigate the effect of incorporating research-based activities into Studio Physics on students' conceptual learning gains.

1.2.6 Technology:

Microcomputer-Based Labs(MBLs), Video Based Lab (VBL), complex three- dimensional simulations, and student response systems (clickers) all are technologies that have been employed in classrooms and show promising results in impacting students performance. For example, offering extensive video motion analysis demonstrations over an extended period of time (R. J. Beichner 1996) to introductory physics students showed that all students performed better in interpreting kinematics graphs than students in traditional instruction classes. A study on using computer simulations (N. D. Finkelstein *et al.* 2005) demonstrated that substituting a computer simulation for real laboratory equipment led to outperformance among students who used the simulation (simulated light bulbs, meters, and wires) compared to students who used real equipments (real light bulbs, meters, and wires). Clickers also have been used in large classes in assessing student' opinions and understanding, and increasing student involvement and instructor-student interaction (J.E.Caldwell 2007).

1.2.7 Evaluation of Specific Instructional Interventions:

A large number of pedagogies have been developed based on (PER) to improve teaching physics. Evaluating the effectiveness of these pedagogies was the goal of this field of (PER). Jeff Saul (Jeffery S. 1998) , for example, compared traditional calculus-based instruction with the University of Washington's Tutorial (www.phy.washington.edu/groups/peg/tut.html), University of Minnesota's Group Problem Solving & Problem Solving Labs (P. Heller & M. Hollabaugh 1992) &(P. Heller *et al.* 1997), and Dickinson College's Workshop Physics (http://physics.dickinson.edu/~wp_web/wp_homepage.html). The (FCI) & (MPEX) mentioned earlier were used as part of the research methods used in the comparison. The main results of the study suggested that the research-based methods are more effective in improving conceptual

understanding, and showed relatively improvement in student's expectations than the traditional method. In another study, Interactive lecture Demonstrations (ILDs) were evaluated (D. Sokoloff & R. Thornton 1997) and have been found to be very successful for teaching physics concepts.

1.2.8 Instructional Materials:

In its attempts to make teaching and learning physics more effective, the (PER) community produced includes research-based textbooks and supplements that are now widely used. Physics by inquiry (*Physics by Inquiry*, Lillian C. McDermott *et al.* 1996) is a model for these books. The book is a result of more than 25 years effort of the Physics Education Group at UW. It is a set of laboratory –based modules providing smooth introduction to physics and physical science. Physics for Science and Engineers (*Physics for Scientists and Engineers*, R. Serway *et al.* 2000) is one of the first physics textbooks with a structure based on (PER) findings. Also, Knight's textbook of the same name (*Physics for Scientists and Engineers*, 2004) is the most widely used PER-based text.

1.3 The focus of this Work:

As mentioned earlier, the motivation of (PER) at the University of Washington was the recognition of students' difficulties with basic concepts in physics .Consequently, the Physics Education Group at the university started investigating the roots of these difficulties. These investigations along with others around the US were behind all the above reported numerous innovations in all research areas of (PER) that led to significant improvement in teaching and learning physics. As such, recognizing “what is wrong “is a necessary starting point for drawing the ways to the solutions. Since my goal when choosing Education Research was implementing the findings of (PER) in the US to improve the quality of teaching physics back home, having a tool to diagnosis the current instruction then evaluating the planed one was apriority in achieving that goal. The Translated Conceptual Survey of Physics (TCSP) was the invented tool for our purpose.

CHAPTER 2

METHODS

This chapter is devoted to explain how the Translated Conceptual Survey of Physics (TCSP) was designed, translated, and validated. My intension to use the (TCSP) back home in Libyan schools suggested two things: first, rather than writing it from scratch; we would combine questions from established tests that have been tested for its validity and reliability (see below the definition of a test validity and reliability). Second, we constructed the test to cover as much as possible of physics introductory topics by choosing questions about force and motion, and electricity and magnetism – that are appropriate for introductory physics students back home.

Validity and reliability are two overall measures of a test quality. **Validity** is an estimate of how well the test measures what it attempt to measure (see (Maloney, D. *et al.* 2001) for more details on this). **Reliability** is a measure of how consistent the test under the same conditions (see the above reference).

2.1 Questions Selection:

The test consists of 31 questions. Questions 1-15 are in Newtonian mechanics, while questions 16-31 are in electricity and magnetism. Questions 1&2 and questions 7-15 are adopted from (FCI), with a minor change in the wording of the first line in Q 1 in attempt to make it more clear in response to our students interviews. Questions 3-6 are constructed based on ideas from questions 14-21 & questions 22-26 in the (FMCS). Questions 16, 17, 30, and 31 are adopted from Conceptual Survey in Electricity and Magnetism (CSEM) (Maloney *et al* 2001), with the answer option (E) in questions 16& 17 is changed from “other” to “none of the above”. Questions 18-29 are adopted from a Brief E &M Assessment (BEMA) (Chabay & Sherwood 1997), with questions that have more than five answer options were reduced to 5 options (A-E). Tables 2.1 and 2.2 below show the mechanical and E & M concepts included in the test.

2.2 Translation Process:

When translating the survey to Arabic to use it to assess students back home, three aspects was considered; the first aspect is related to the topics that are covered—as mentioned above-

Table 2.1: Newtonian concepts in the test:

Mechanics Concepts	Test Item
1. Kinematics: Graph interpretation (Velocity & Acceleration). Velocity discriminated from position.	Q3-Q6. Q12. Q8.
2. First law	
3. Second law : Constant force.	Q1 & Q13.
4. Third law.	Q2, Q11 & Q14.
5. Superposition principle: Vector sum of velocities.	Q9.
6. Kinds of forces:	Q7, Q10 & Q15.

Table 2.2: Electricity and magnetism concepts in the survey:

E & M Concept	Test item
1. Coulomb's law: Force & charge. Force & distance.	Q16. Q17.
2. Electric force on a point charge.	Q18.
3. Magnetic force : on a point charge. on a neutral metal bar. From a carrying current wire.	Q24 & Q25. Q30. Q27.
4. Polarization of dielectric materials.	Q19.
5. Gauss's law.	Q23.
6. Magnetic field due to carrying current loops.	Q26.
7. Capacitors.	Q21.
8. Potential difference.	Q20 & Q22.
9. Faraday's law.	Q28, Q29 & Q31.

which have to be appropriate for students backhome. Most of the topics in the survey are introduced in High school and then are taught again –in more depth- in introductory physics college classes. The second aspect is the wording of the problems. When considering this aspect;

all physics concepts and other scientific concepts in the problems are translated to the familiar Arabic concepts that are commonly used in classrooms there. For example, "Velocity is translated to سرعة", "Potential difference to فرق الجهد", "Exerts to يؤثر", and etc. Under the second aspect also, the notion of keeping the whole meaning of the problem in Arabic as it is in English, which is different from translating word by word. The third aspect is the situation in which the problem is introduced. The situations the problems involve, such as a truck colliding with a compact car, a woman pushing a box, a proton or/and electron passes through a region of electric/magnetic field, etc.. are all familiar situations in both cultures and should not affect attempts to understand the problems. Therefore no changes of this sort have been made to the problems, with the exception of problem 9, where a hockey puck is used as the object in the question. But since "Hockey" is not a familiar game in Libya; the term "metal disk" is used instead to avoid any confusion.

2.3 Test Validity:

As stated previously, the questions in the test are adopted from tests where a considerable care was taken to establish the validity and the reliability (R. K. Thornton & D. R. Sokoloff 1998) & (Ding, L. *et al* 2006). Even so, we interviewed four students (three English speaker students & one Arabic speaker student) to make sure that the TCSP as written was being clearly interpreted – especially for questions 3-6. Each interview was around an hour long. Students read the question then explained what they thought it was asking about and, if appropriate, made some suggestions about the problem text that would make it more clear for them. In general, and when the whole question was read, students showed an understanding of what they were asked - whether they got the right answer or not. Two of the English speaking students exhibited a good interpretation in a short time of most of the questions and a successful recalling of the relevant physics contexts when deciding which the right answer is. However those two students stopped for a while at Q3. By reading the introduction to Q3-Q6 and looking at the graphs students were confused and struggled in understanding what the questions are asking, and they suggested that the Y axis should be labeled. But once they read Q4 and saw the "big picture" they were not confused any more. The third English speaker student spent longer time-relatively- in reading most of the questions and trying to understand their intent. Beside the same confusion about the Y axis in Q3-Q6 he also seemed to be hesitant about interpreting some physics concepts in these

questions such as “speeding up at steady rate” as either constant speed or constant acceleration. Even when he made a correct interpretation, he hesitated in deciding the relevant graph. The confusion Q3-Q6 caused before reading the whole question suggested adding the statement “read the whole question before answering” at the end of the introduction to the mentioned questions. A minor change was made to Q12 too by labeling which is block A and which is block B. Students suggested it would facilitate understanding the associated figure. The wording of the rest of the Newtonian mechanics questions and the E&M questions did not seem to cause students to trouble with interpretation.

The fourth student was a native speaker of Arabic. He was interviewed with the translated version of the survey to see if the Arabic text of the problems is clear. None of the problems caused confusion for the student, and the student’s interpretation matched the interviewers. Some comments were made regarding some concepts that might be translated differently in different Arabic regions such as Acceleration which could be translated to "عجلة" or "تسارع", Capacitor; could be translated to "مكثف" or "متسعة". However, other words in the problem text or the associated figures make the meaning of such concepts clear. Hence, the whole problem was still understood.

CHAPTER 3

DATA ANALYSIS

The (TCSP) was administrated to PH100 & PH200 students at the CSM as a pretest, before any classroom instruction. The PH200 pretest can be considered to be nearly equivalent to a PH100 posttest. Below is a description of the most recognized features in students' answers and the statistical techniques used.

3.1 Data Analysis for PH100 students:

The collected data can be looked at based on different aspects. Our first comment on the data was based on the number of students who got the right answer for questions that cover the same concept. Some features were noted under that aspect in the Newtonian mechanics part as follow:

for **Q2, Q11 & Q14** which cover Newton' third law in different situations; more than half of students (51 out of 78) chose the option that states that “the truck exerts a greater amount of force on the car than the car exerts on the truck” for Q2 –where both the car and the truck collides ahead. While on Q11 – where the truck is pushed by the car- more than half students went with the option “the amount of the force with which the car pushes on the truck is greater than that with which the truck pushes on the truck”. On Q14 almost half students answered that the student with the greater mass exerts greater force on the other student. These answers seem consistent with common misconceptions identified by the (FCI) in table II in (D. Hestenes *et al* 1992).

On **Q3-Q6** which covers velocity and acceleration graphs; more than half students population (62 out of 78) got the e right answer (e) for both questions (Q3&Q5) which is asking for selecting a velocity versus time graphs. On Q4 &Q6 which are looking for acceleration versus time graphs; even though students who got the right answer were more than half students population on Q6 and a little less than that on Q4, there were a considerable number of students who chose the options that would be the right answer for each question if the required graphs are velocity versus time (that might reflect situations of not reading the question carefully).

Q10& Q15 which test students understanding of gravitational force, air force and force received by a hit (an impulse). More than half students' choices on both questions might reflect

another common misconception- that a force revived by a hit is still acting on the object even after losing contact.

Q13 covers Newton second law. More than half students chose that “the force exerted by the women is grater than the total force which resists the motion of the box” most likely interfering that from the motion of the box – again another common misconception.

Students’ answers to the E &M part -in general – do not reveal any thing specific, since it was distributed randomly between the choices (as one would expect from a pretest). Only on **Q16** which covers Coulomb law and **Q23** which covers Gauss’ law did more than half of the students get the right answer (66 & 40 students respectively). On **Q17** which also covers Coulomb law more than half students chose the option that reflect that the relation between the force(F) and the separated distance (r) is $(F \propto 1/r)$ instead of $(F \propto 1/r^2)$.

3.2 Data Analysis for PH200 Students:

In this section the features observed in (100) PH200 students answers are stated along with a comparison with PH100 students’ performance on the same questions (a more accurate quantitative comparison is in the next section).

Q2,Q11 & Q14: most students got the right answer on Q2 & Q14, while more than half students got the right option on Q11. A considerable number of them chose the option that the car exerts a larger amount of force. This reveals a measurable improvement comparing to PH100 students’ performance.

Q3-Q6: better performance than PH100 is noted on this group of questions -where again most students got the right answer.

On **Q10 & Q15:** almost the same number of students (74 & 75 students on Q10 & Q15 respectively) selected the correct option, while most of the rest went with the option that counts for the force received by the hit. This performance is almost opposing PH100 students’ performance on the same questions.

On **Q13** even though more than half students (54) got the correct answer, a considerable number of them (38) selected the option that states that the force exerted by the women is greater.

On the E & M part only on questions **16 & 17** most students answered correctly (91&68 students for each question respectively).

On **Q19** more than half students think that the rubber sheet will not be affected by the charges on the wall since it is an insulator. Looking back to PH100 responses to this question will reveal some thing interesting, where more than half students also were distributed between the two choices that state that rubber sheet will not be affected either because of the above reason or because it has a zero net charge.

Looking only at the number of students who got the correct choice for the rest of the questions might not be statistically meaningful, where that number was in the forties for questions **20-23**. Also for **Q24,Q25 &Q30** and **Q28 & Q29** -where answering correctly will require recalling the same concepts and formulas for each group- the number of students answering right was not statistically meaningful.

3. 3 Statistical techniques used:

We used a two-tailed z-test to compare the mean of the two populations (Neil A. Weiss 1995).

The purpose of using the z-test is to test the hypothesis that (PH200 students perform better than PH100 students in each of the two parts of the test) based on the data collected from the two samples.

The mean (\bar{x}) and the standard deviation (σ) of each sample in each part of the test were calculated using Excel as shown in table 3.1.

Table 3.1: The mean and standard deviation for PH100 and PH200 students

	PH100	PH200
Mechanics	$\bar{x}_1 = 7.231$ $\sigma_1 = 2.941$ $n_1 = 78$	$\bar{x}_2 = 10.97$ $\sigma_2 = 3.145$ $n_2 = 100$
E&M	$\bar{x}_1 = 4.167$ $\sigma_1 = 1.418$	$\bar{x}_2 = 5.26$ $\sigma_2 = 1.835$

From table 3.1 and using the formula:

$$Z = (\bar{x}_1 - \bar{x}_2) / \sqrt{((\sigma_1^2 / n_1) + (\sigma_2^2 / n_2))} \quad (3.1)$$

The value for the z- test statistics for the mechanics part is:

$$Z < 0.001$$

This is the area under the standard normal distribution of the difference of the sample means ($x_1 - x_2$) that corresponds the value calculated from equation (3.1) – the P value. P value can be obtained from Table II in (Neil A. Weiss 1995).

For the E&M part Z is also < 0.001 . This means the test results (for both parts) are statistically significant and PH200 students did perform better on both parts than PH100 students. This performance was expected on the mechanics part where PH200 students have taught the relevant materials prior taking the test, but PH100 students have not. While on the E&M part where both groups was not taught the relevant materials prior taking the test , it might be surprising that PH200 performed better- even though the difference was very small, and rendered significant mostly by the very large sample sizes.

CHAPTER 4

CONCLUSION AND POSSIBLE APPLICATIONS BACKHOME

This chapter is devoted to discuss the possible applications of the test back home in an aim to improve the quality of teaching physics there.

4.1 Review of the Test Data:

Looking back to the test data obtained in chapter (3) it can be concluded that the test runs normally with out any surprising responses. Where the responses of PH200 students –who had taught the relevant topics prior taking the test- to the Newtonian part, were based on mechanical thinking and the students performed well. PH100 students – who had not taught the relevant topics-, based most of their answers (to the same part) on common sense and performed scientifically less well. On the E&M part, however, both groups' answers distributed randomly between the choices – that is expected since both groups had not taught the relevant topics prior taking the test.

According to the above discussion – and taking in account that the test questions were adapted from known tests that were conducted widely across US- the survey can be taken home to assess teaching and learning physics there (where using such surveys is rare –if surveys are used at all). The next section discusses how the main applications of such surveys in US can be used back home.

4.2 Applications of the Test Back Home:

The main goal of this work – as stated in chapter 1- is applying interactive teaching pedagogies to improve teaching physics back home. It is thought that having a tool -to diagnose the current situation and use to evaluate a suggested form of instruction- would be the right step. Application of the TCSP while teaching college physics can be in three categories (D. Hestenes *et al.* 1992):

(1) **As a diagnostic tool**, the test problems and multiple choice answers were designed in a way that stimulates common forms of student reasoning. Interviewing students will then be a good way to learn how they think about the topic, and way to uncover misconceptions. Interviewing techniques for mechanics and E&M in the US are well- established (L. McDennott

1984). A challenge in conducting the survey back home, however, would be that most students are not familiar with such surveys or interviews. This suggested that students should be familiarized with the test by giving them previous similar test format and discussing it in class. It should be interesting to compare misconceptions among students at US and back home.

(2) For evaluating instruction, hopefully, the TCSP would be a start of a series of other surveys that assesses student's understanding of other physics topics to evaluate and hence, improve teaching physics in general. Since the test problems were taken from known tests that assess student's overall grasp of mechanics and E&M, giving the survey as posttest should be an indication of how well a course was taught. Collecting pretest and posttest data over a period of time from conducting such surveys – at the beginning and the end of both traditional and interactive instruction- will reveal wealth information for research purposes. Quantitative evaluation and comparison between the two kinds of instruction should provide convincing evidence that the performance differences between courses is mainly a result of that instruction.

(3) As placement exam, the test can be used to determine how much student understanding and retention of introductory physics concepts is sufficient for more advanced courses.

CHAPTER 5
INTRODUCTION TO TWO PHOTON EXCITATION
FLUORESCENCE MICROSCOPY

Because of its properties, two photon excitation fluorescence **TPEF** microscopy is the preferred imaging approach in life science. Safe and deep imaging of up to 1mm in living animals and intact tissues has been done. Advances in the techniques of implementing **TPEF** microscopy in imaging living moving systems will benefit the field of biology and biomedical research.

5.1 What is two photon excitation fluorescence (TPEF) microscopy:

TPEF microscopy is based on the idea of two photons of longer wavelength being absorbed in a single quantum event by the fluorophore molecule to emit a fluorescent photon at higher energy than either of the absorbed photons. The phenomena of two photon absorption was predicted in (1931) by Maria Goeppert Mayer, but was not observed until (1961) (Kaiser& Garrett), after the invention of laser due to the high excitation intensity requirement. The first utility of **TPEF** in laser scanning microscopy **LSM** was in (1990) by Winfried Denk. Since in **TPEF** two photons are being absorbed simultaneously, the transition probability depends on the square of the excitation intensity. Focusing and the use of a pulsed laser, therefore, increase the probability of two photon absorption, and makes **TPEF** microscopy possible. A mode-locked titanium sapphire (Ti: Sapphire) crystal laser –that produces pulses of (~100 femtosecond) duration for each, and at repetition rate (~80 MHz) is the most common used laser in **TPEF** microscopy (or in multi photon microscopy **MPM** in general) ,with excitation wavelength in the near infrared **NIR** range (750 -1100 nm).

The squared dependence of the excitation probability on the excitation light intensity is essential for the localized nature of **TPEF** microscopy, resulting in photobleaching and photodamage that is restricted to the focal plane. In contrast to one photon excitation microscopy, where bleaching occurs through the focal volume. Localization of excitation also provides 3-D resolution with no pinhole is required. This means scattered photons will not be blocked, and will contribute to the signal, increasing image quality. The **NIR** wavelength regime is scattered less than the shorter wavelengths used in confocal microscopy and hence, penetrates deep in tissue (>100 μm), and it has been used to image to depths of up to (1mm). Longer wavelengths are also more tolerable to living systems. These advantages of **TPEF** microscopy is behind its preferable use in biology.

5.2 Applications:

The following sections illustrate a summary of the applications of **TPEF** microscopy in some areas of biology, showing how these areas benefited from the advantages of this nonlinear fluorescence microscopy.

5.2.1 Developmental Biology:

The utility of **TPEF** microscopy in many applications of developmental biology has shown better results than conventional microscopy. Lineage tracing of sea Urchin embryo (Summers *et al.* 1996) was the first example of using **TPEF** for lineage tracing. In this study the caged dye (fluorescein-dextran) was uncaged using two photon excitation microscopy which was less damage to embryos than physical or electrical perturbation.

Also, an evaluation of **TPEF** versus Confocal and digital deconvolution fluorescence microscopy imaging in the frog *Xenopus* morphogenesis (the biological process that causes an organism to develop its shape) (Periasamy *et al.* 1999) has shown significant advantages of **TPEF** compared to the other two techniques in terms of image contrast and the absence of background fluorescence.

In another study of deep genetic analysis of pancreatic development (Huang *et al.* 2001), **TPEF** was used to generate 3-D reconstructing images for living zebrafish embryos -which has been established as a popular vertebrate model. These were some examples of applying **TPEF** microscopy in developmental biology in an effort to address unanswered question in the field.

5.2.2 Calcium Imaging:

The highly localized photorelease of caged compounds- such as **Ca²⁺**- associated with **TPEF** (Denk *et al.* 1990) has been used to study cell signals as well as in developmental biology. This is especially of great importance in scattering tissues –like brain tissues, where the less scattered **NIR** long wavelengths penetrate deep even within such tissues. Using **TPEF** in imaging cerebellar Purkinje cells, it was proved that the fundamental unit of neuronal integration is the single spine rather than the spiny branchlet (Denk *et al.* 1995).This is crucial for neuroscientists to understand neuronal activities.

Mainen and others (Mainen *et al.* 1999b) described the use of **TPEF** to image transients increases in **Ca²⁺** concentration in postsynaptic receptors in a single spine for a direct test of receptors saturation. They imaged fluorescence transient in rat hippocampal slices to show that the receptors are not saturated by a single release of glutamate molecules from a vesicle.

In intact retina, **TPEF** microscopy- in contrast to conventional microscopy was used as a safe technique to measure calcium concentration transients without blocking visual stimuli due to excitation of photoreceptors. Consequently, this enables researchers to study calcium signals induced by natural stimuli (Denk *et al.* 1999).

It is worth to mention that intracellular calcium signals are important in understanding functional state of cells, and the measurement of these signals with **TPEF** requires synthetic calcium indicators via electrodes.

5.2.3 *In vivo* Imaging:

With its deep penetration and localized nature, **TPEF** microscopy has become the tool of choice for imaging inside living whole animals. This gives the advantage of studying cell functional and developmental changes in their natural environment. In addition to some of the above examples in developmental biology and calcium imaging that were done in intact animals, below are illustrations on a couple more.

In a study of capillary blood flow in olfactory bulb glomeruli, Wistar rats were scanned with **TPEF** over the desired region with the aim of understanding the coupling between neuronal activity and cerebral blood flow. Two photon microscopy allows the measurement of red blood cell flow with high spatial and temporal resolution (Chaigneau *et al.* 2003).

In another example- among neurobiology, two photon imaging was used to demonstrate that hippocampal long-term synaptic plasticity is associated with dendritic spine changes. Using this novel technique enables observations of postsynaptic dendrite to the level where synaptic changes occur (Engert & Bonhoeffer 1999). Also, long term imaging of living mouse by **TPEF** was done to understand long term changes in adult and developing animal spine (grutzendler *et al.* 2002).

The high 3-D resolution of **TPEF** microscopy plays an important role in its utility in pathologies for better understanding and hence, treatment purposes. Measurements of gene expression and

physiological functions deep in individual tumor cells have been shown using the two photon imaging approach. Microscopic structures that can be used in diagnosing Alzheimer disease were imaged up to (150 μm) in living mouse using **TPEF**. These images were used to observe the effect of immunotherapy on the disease (Bacsikai *et al.* 2001).

5.3 The Focus of This work:

The introduction of **TPEF** to the field of microscopy has its significant advantages especially in imaging thick tissues and living systems. The use of short laser pulses is an important factor to obtain sufficient signal. Currently, self-mode-locked titanium: sapphire (Ti: Sapphires) laser (Spence *et al.*, 1991) is the common excitation sources for **TPEF** microscopy. The wide range (~750- ~1100) provided by this source gives an access to most fluorophores used in biology. A limiting factor of **TPEF** microscopy, however, is the cost of the excitation source (> 150,000 \$) (Denk *et al.* 2006). Mode locking techniques were introduced to work on this issue, but they were either complicated, still expensive, or not of practical application (Keller, 1994).

Even though short pulses increase the probability of two photon absorption-as stated previously, relatively short pulses (>100 fs) may result in group velocity dispersion (GVD) .This effect reflects the fact that short pulses consist of wide range of wavelengths that travel with different speeds within the microscope optics. This leads to a chirped pulse (pulse that is longer than the original pulse).Group dispersion can be compensated-if it is worth the effort, or if conventional microscopy is limited by the depth or by its damage to the sample under investigation- by pre-chirping using a prism or grating arrangement (Fork *et al* 1984).

Although confinement of excitation to the focal plane in **TPEF** microscopy restricts photobleaching and photodamage to that plane, damage and bleaching may be considerable for some fluorophores that bleach more easily, or when there is a high concentration of fluorophore molecules. Photodamage and photobleaching associated with **TPEF** microscopy has been extensively studied, see for example (Hopt, A.& Neher, E. 2001)and(Paterson, G.H. & Piston,D.W 2000). It is important to understand the properties of the biological system under investigation, and minimizing excitation intensity as much as possible for safe use of **TPEF** microscopy.

Aberration is another effect that presents itself in **TPEF** microscopy. Aberrations result from focusing through layers with different refractive indexes, like imaging in thick tissues. This

prevents tight focus of the laser beam which, consequently, degrades image quality, and lower excitation efficiency. Adaptive optics have been used in different strategies to correct these aberrations. Some of these strategies involve compensation of specimen-induced aberration within a specific depth (Niel, M.A. *et al.* 2000), or others for dynamic compensation of aberration, which can correct aberration during scanning within different depths (Sherman L. *et al.* 2002). Also, water-immersion objective lenses have been used to avoid aberration in aqueous specimens (A. Egner & W. Hell 2006).

In addition to the above mentioned limitations of **TPEF**, its powerful use for imaging living systems introduces another challenge, which is the motion of these systems. The Remote focusing technique- that has been introduced as an alternative to the mechanical movement of the specimen relative to the objective lens, when optical sectioning from a range of focusing depths is required (J. Botcherby *et al.* 2007) - could be coupled to a microcontroller circuit to develop tracking system that keeps the focal plane stationary, which is the focus of this work.

CHAPTER 6

REMOTE FOCUSING TECHNIQUE

This chapter is devoted to review the state of the remote focusing technique and summarize the description of a remote focusing system that we think, by receiving electric feedback; can track a desired focal plane within a living, moving animal. But first, a description of other focusing strategies –that led to the need of such technique- is given.

6.1 Refocusing Techniques in Microscopy:

Generating 3-D images of a desired biological system is a powerful aspect of confocal and multiphoton microscopy as these images reveal a wealth of information about the system under investigation. A series of images from different focusing depths are generated via refocusing the microscope. These images are then processed on a computer to obtain three dimensional image. Since for high numerical aperture (NA) microscopes the focal plane and the image plane are well defined, refocusing the microscope cannot be done by moving the detector along the optic axis. Instead, refocusing techniques that have been introduced involve changing the distance between the objective lens and the specimen. Below is an elaboration on some of these and drawbacks associated with them.

Callamaras (N. Callamaras & I. Parker 1999) constructed a real-time laser-scanning confocal microscope that allows axial (x-z) imaging using a piezoelectric focusing unit (P-721.10; Polytec PI Inc.) to focus the objective. Even though the system was simple and easily built, the axial scanning speed is still limited by the objective inertia. Also, the movement near the specimen might disturb it.

The high speed focusing requirement was behind another focusing technique that relies on a lens that changes its shape. One of the studies that uses this concept and was able to achieve 1-KHz bandwidth was introduced by (H. Oku *et al* 2004). A lens that deforms its shape by a pressure applied to a transparent liquid was used. Although 1-KHz bandwidth is an adequate for high speed focusing, this method suffered from aberrations.

In another study aiming to refocus remotely, a microelectromechanical MEMS mirror was integrated into a high speed optical coherence tomography (OCT) system by (B. Qi *et al.* 2004) to design a system that can be used to shift the focus plane of the sample beam simultaneously with the depth scanning of the coherence gate. With the improvement to the image quality that researchers demonstrated, they anticipated that by increasing the system NA and the MEMS mirror deformation, the system will be compatible with endoscopic imaging.

6.2 Remote Focusing Technique:

An alternative focusing method, that overcame most of the above mentioned problems, was introduced by (E. J. Botcherby *et al* 2007). Refocusing in this method is implemented remotely without mechanical movement near the specimen so it avoids aberration and shifting the specimen which may affect the process under investigation. It also enables axial scanning speed on the order of milliseconds. Figure 6.1 shows the architecture of a remote focusing system. The system comprises of $4f$ imaging system that is built using two microscopes- back to back – with two high matched NA objective lenses (L1, L2) and two achromatic doublet lenses.

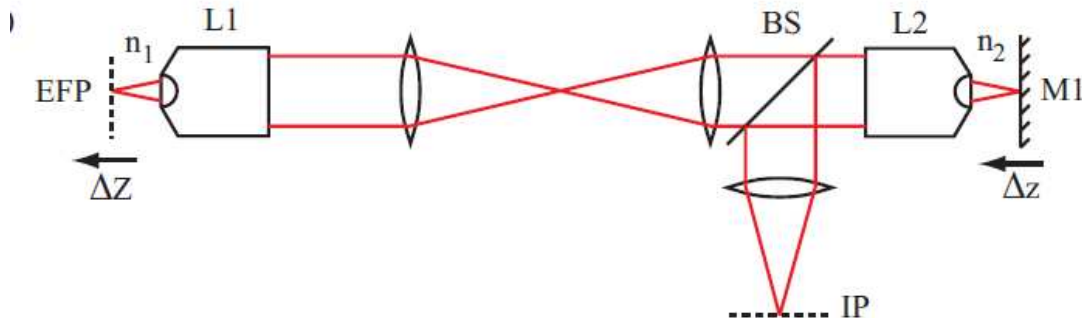


Figure 6.1: Remote focusing microscope (Image taken from (E. J. Botcherby *et al.* 2008 b))

The magnification factor of the $4f$ imaging system has to be:

$$M = \rho_2 / \rho_1 = \sin \alpha_1 / \sin \alpha_2 \quad (6.1)$$

Where ρ_1 & ρ_2 are the normalized pupil radii of L1 & L2 and α_1 & α_2 are the semi aperture acceptance angles of L1 & L2 respectively. This system represents a perfect imaging system with a unit magnification factor (E. J. Botcherby *et al* 2008 a) where any aberration introduced by L1 is canceled by that introduced by L2 when focusing onto different depths within the specimen. As a result, a diffraction-limited image will be formed at the focal plane of L2. Detecting this image, however, requires a detector with a resolution that is a fraction of a wavelength. So, a third microscope is used to magnify this image. The plane mirror m1 shown in figure 6.1 is mounted on a piezo scanning stage and placed at the focal plane of L2 to reflect the image back into L2 which in this case works as a part of the third microscope. The beam splitter and the quarter wave plate are used to further direct the rays. Refocusing in this system is carried out by changing the axial position of m1 (moving m1 toward L2 distance Δz will shift the focal plane deeper in the specimen by: $(\Delta Z = (2n_2/n_1) \Delta z)$). Since the mirror lies in the focal region of L2, it can be very small which allows high axial scanning speed comparing to moving the specimen or L1, and further it is done without introducing aberration or disturbing the specimen.

6.3 Measuring the Point Spread Function:

In this section, the definition of the point spread function (PSF) is introduced. Then followed by the results for the measured (PSF) once for a system that refocuses using traditional technique, and another for a system that refocuses using the new remote focusing technique.

In a perfect imaging system, each single point in the object space re-converge to a single point in image space (E. J. Botcherby *et al* 2008 a). However, in real imaging, each point of the objective is replaced by a blurred point (or a diffraction limited spot) in the image plane. The (PSF) is the image of a single point (i.e. PSF is the diffraction limited spot) (Periasamy *et al* 1999). Since the (PSF) describes the blurring of an image that is introduced by the microscope, measuring it would be a key metric of microscope performance.

For the traditional refocusing method, the confocal configuration shown in figure 6.2 was used (E. J. Botcherby *et al* 2008 a). The system comprises of an objective mirror (M1) placed a distance (z) from the focal plane of L1, an Olympus 1.4NA 60x oil immersion objective lens (L1), an achromatic doublet tube lens with focal length 160 mm, CCD camera, and a beam splitter. Refocusing was done by moving the detector (CCD camera) axially. The measured (PSF)

for different positions (Z_c) of the CCD camera is shown in figure 6.3a where the effect of the spherical aberration can be clearly seen.

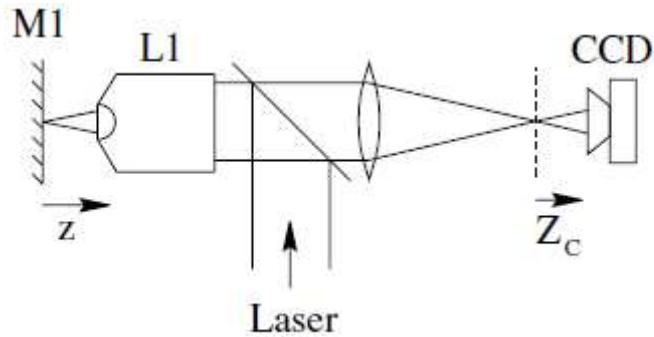


Figure 6.2: Traditional refocusing method (Image taken from (E. J. Botcherby *et al.* 2008 a))

For the remote focusing technique, the same system in figure 6.1 was used (E. J. Botcherby *et al.* 2008 b). Where L1 with the same characteristics as above, L2 is an Olympus 0.95 NA 40x dry objective, the first two tube lenses are achromatic doublet with focal length 160 mm each, and the third tube lens is an achromatic doublet with focal length 200 mm. The (PSF) was measured again for each position of M1 in figure 6.1, and the measured (PSF) is shown in figure 6.3b. Comparing this to figure 6.3a it can be seen that there was no a significant affect of aberration on the shape of the PSF when focusing onto deferent depths (-30, -20, -10, 0, 10, 20, 30).

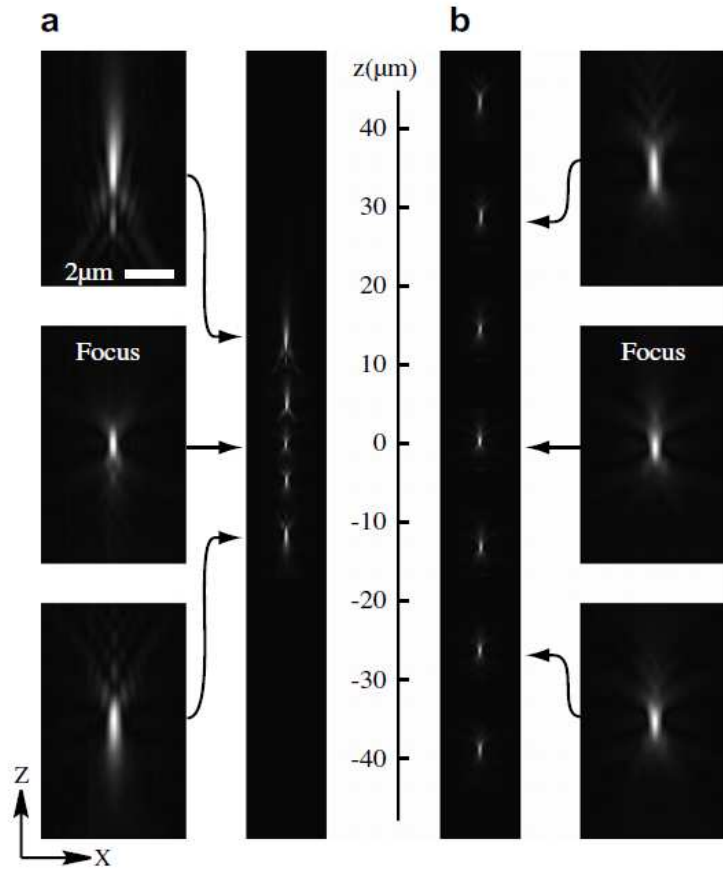


Figure 6.3: PSFs measured for system refocuses via: (a) Traditional technique, (b) Remote focusing technique (Image taken from (E.J. Botcherby 2008 a)).

CHAPTER 7

INTRODUCTION TO MICROCONTROLLERS

The electric feedback that will be sent to the remote focusing system –as mentioned in chapter 6- is regulated through a microcontroller. This work uses the Arduino platform for prototyping which features the ATmega328 microcontroller. The advantage of this system is that it is entirely open source. Programming is achieved through C coding through an intuitive interface based on the processing environment developed at the Massachusetts Institute of Technology. The basics of the microcontroller are described in the following sections.

7.1 Arduino Uno Board:

There are many types of Arduino boards with different features ([http:// www.arduino.cc](http://www.arduino.cc)). Arduino Uno revision 2 is the one was used in this work. Arduino Uno is the latest revision in the series of USB Arduino boards, and it features the ATmega328 microcontroller. As shown below in figure 7.1 the board has 14 digital pins(0-13) that can be used as input/output using `pinMode()`, `digitalWrite()`, and `digitalRead()` functions – 6 of these pins support pulse width modulation (PWM) output (see below), 6 analog inputs(A0-A5), a 16MHz crystal oscillator, USB connection, power jack, In-Circuit Serial Programming(ICSP) header (see the programming section), and a reset button.

In addition to input/output, some of the 14 digital pins have other special functions such as:

Serial communication: Pins 0 & 1 can be set to receive and transmit serial data.

External interrupts: Pins 2 &3 can be used as interrupts. Interrupts enable the Arduino to monitor an external event and automatically return to doing its normal program. The function `attachInterrupt()`-which takes 3 parameters - is used for this purpose. The first parameter determines which pin (2(interrupt 0) or 3 (interrupt 1)) to monitor. The second parameter is the location of the code that is needed to be executed while monitoring the event. The third specifies what type (mode) of trigger to track (low value, falling or rising edge, changing value).

PWM 3, 5, 6, 9 &10. These pins can provide an analog output using a digital signal can be done by controlling the time that the signal spends on (5 volts) or off (0volts)(i.e. modulating the pulse width). If that is repeated fast enough, the result will be a simulated voltage between (0 V



Figure 7.1: The Arduino Uno board.

(0% duty cycle) and (5 V) (100% duty cycle) as illustrated in the plot below in figure 7.2. The output value can be set using the `analogWrite ()` function. The function takes 2 parameters; the first determines which pin is used, while the second parameter takes a value from 0-255.

Even though the **analogWrite** function can change the time that signal spends on or off, there is another factor that controls how fast PWM is. That is the default frequency for each pin, which is around 1000 Hz on pins 5 & 6 and about 500 Hz on the other PWM pins. Changing PWM frequency is achieved via controlling three timers- one timer for each pair of PWM pins. Timer 0 is used to control pins 5&6, timer 1 is used to control pins 9 & 10 and timer 2 is used to control pins 3 & 11. Each one of the three timers has specific settings (i.e. frequencies). The format of the function used for this purpose is as follows:

`TCCR0B = TCCR0B & 0b11111000 | (setting);` for pins 5 & 6. Where the settings can be 0×01 to 0×05 with each one indicating a different frequency.

`TCCR1B = TCCR1B & 0b11111000 | (setting);` for pins 9 & 10. With settings from 0×01 to 0×05 .

`TCCR2B = TCCR2B & 0b11111000 | (setting);` for pins 3 & 11. With settings from 0×01 to 0×07 .

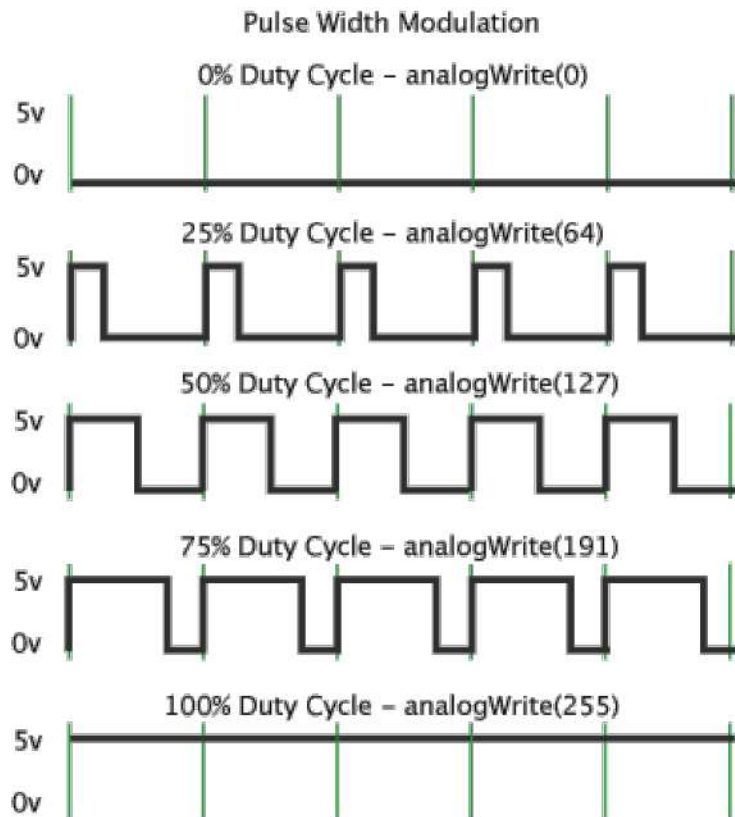


Figure 7.2: Pulse width modulation (Image taken from (www.arduino.cc/en/Tutorial/PWM))

SPI: 10(SS), 11 (MOSI), 12(MISO) &13(SCK). These pins are used by the Arduino- which in this case is called Master- to communicate with one or more peripheral devices –which is called the Slave- or another microcontroller quickly over short distances. The Arduino uses Serial Peripheral Interface (SPI) protocol for these communications. Slave Select pin (SS) is used by the master to select which device to communicate with. Master Out Slave In (MOSI) is for sending data to the slave. Master In Slave Out (MISO) is for sending data to the master by the slave. Serial Clock (SCK), is used to synchronize data generated by the master.

Some of the six analog pins have specialized functions as follows: **TWI: A4 SDA & A5 SCL.** These pins enable the Arduino to communicate with two-wire interface (TWI) devices through the data line (SDA) on A4 and the clock line (SCL) on A5. The wire library is used for these communications.

Power pins **VIN**. If the Arduino is powered by battery, leads from the battery can be connected to GND & VIN pins. If the Arduino is powered from the power jack, VIN can be used to access this power. **5V**. When supplying voltage from USB connector, it can be accessed through this pin, and it is a regulated 5V. **3.3V**. This pin generates a regulated 3.3 volt supply. **GND**. Ground pins.

In addition, there are: **AREF** pin, which is used to configure the upper value of the analog input range using the `analogReference()` function. The function takes one parameter that has these options for Arduino -ATmega328-: **DEFAULT** 5volts for 5V Arduino board or 3.3 volts on 3.3 V Arduino board. **INTERNAL**, which sets the reference to 1.1 volts.

EXTERNAL, this option can be used if an external voltage (0V-5V) is applied on AREF pin. **RESET** pin. Setting this pin to **LOW** will reset the Arduino to the initial state as defined by the user supplied code.

7.2 Arduino Capability:

The Arduino board can be supplied with an external voltage of (6-20) volts. However, the recommended range is (7-12 volts). Working within this range will insure to not drop to less than five volts when connecting to the 5 V pins, and save the board against any overheating that might occur when exceeding 12 volts. Each input and output pin can provide or receive a maximum of 40 mA of DC current. While the DC current from the 3.3 V pin is 50 mA.

The ATmega328 -which is the microcontroller of the Arduino we used- runs at 16 MHz and has a flash memory of 32 KB. 0.5KB of this memory is devoted for the bootloader that enables you to upload a new code simply by pressing the upload button in the Arduino environment. It has also 2KB of static random-access memory (SRAM), and 1KB of electrically erasable programmable read-only memory (EEPROM).

7.3 Programming the Uno Board:

Programming the board is achieved through the Arduino software. Instructions for downloading the software are available on the Arduino webpage. The microcontroller we are using (ATmega328) comes with a bootloader in place, so you can upload new codes with no need to an external hardware programmer-as stated above. Also, an external programmer can be

used to program the board via the ICSP header. Details for this process are again available on (www.arduino.cc).

CHAPTER 8
CONSTRUCTING AND PERFORMANCE OF A PIEZO
CONTROLLING CIRCUIT

This chapter details the construction and performance of a circuit that controls a piezo actuator. The piezo, which is driven by the MDT694A (see below), is used to control the axial position of the scanning mirror in the remote focusing system –as stated previously in chapter 6. Since we want to send an electric feedback to the remote focusing system, we are supplying the MDT694A via the Arduino through an appropriate interface that we constructed based on the features of both the MDT694A & the Arduino.

8.1 Constructing the Circuit:

The MDT694A piezo-electric controller is a single channel (i.e. controls a single piezo) high voltage amplifier. It can provide both manual and external control of the piezo drive voltage. The amplifier has an output voltage switch with three selectable ranges: 0-75 V, 0-100 V & 0- 150 V. The input impedance is 10 k Ω , and the input voltage ranges from 0 to 10 V (www.thorlabs.com). Knowing these output/input specifications for the MDT694A and that the Arduino can supply voltage that ranges from 0 to 5V- as stated in chapter2.3, we know that to access the full range (0-10 V) of the MDT694A when supplying via the Arduino we need an interface with a gain of 2. We used a common emitter amplifier as an interface which is shown below Figure 8.1:

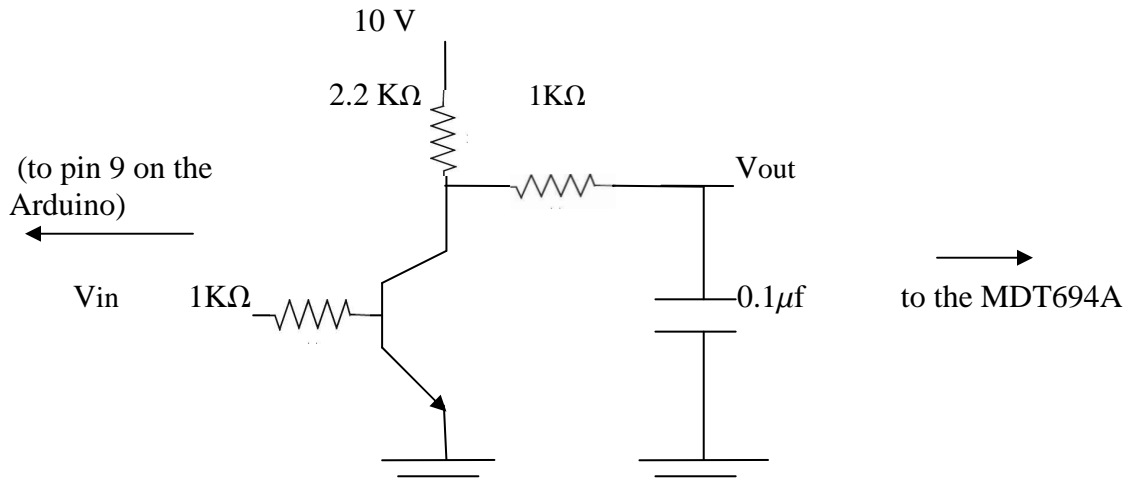


Figure 8.1: Emitter amplifier and low pass filter circuit

We see from the circuit that when:

$V_{in} = 0$ Volt (Turns Off) \longrightarrow $V_{out} = 10$ Volt (Figure 8.2).

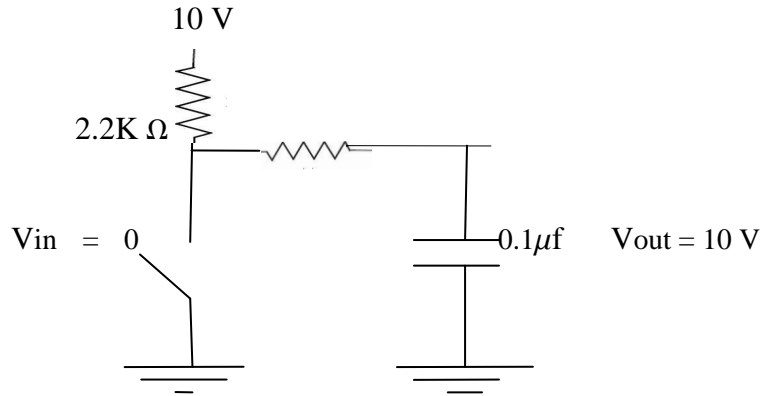


Figure 8.2: $V_{out} = 10$ V when $V_{in} = 0$.

While when:

$V_{in} = 5$ Volt (Turns On) \longrightarrow $V_{out} = 0$ Volt (Figure 8.3).

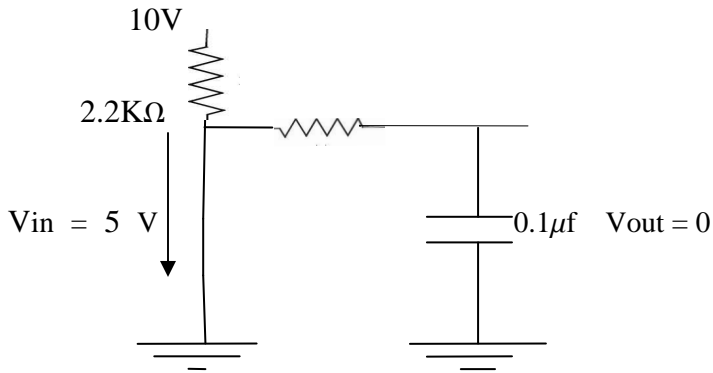


Figure 8.3: $V_{out} = 0$ when $V_{in} = 5$ V.

It is worth to note that the Resistor & capacitor play a filter role in the circuit. It is called a **first-order lowpass filter** where it tends to pass low frequency components and reject high frequency components (in the Circuit performance section, figures (8.7a&b) illustrate the difference on V_{out} with and with out the filter). The filter performance can be explained as follows:

The transfer function of the circuit ($H(f)$) is defined as the ratio of the output phasor to the input phasor:

$$H(f) = V_{out} / V_{in} \quad (8.1)$$

Knowing that the phasor of the output voltage (V_{out}) is the product of the phasor current and the impedance of the capacitor (Allan R. Hambley 2008), we have:

$$H(f) = 1 / (1 + i 2 \pi R C f) \quad (8.2)$$

$$H(f) = 1 / (1 + i f / f_B), \text{ where } f_B = 1 / 2\pi R C \quad (8.3)$$

Now, having the transfer function (and notice that it is a complex quantity)(a complete derivation is available in the above reference). Its magnitude and phase can be calculated to see how that affects V_{out} :

$$|H(f)| = 1 / \sqrt{1 + (f / f_B)^2} \quad (8.4)$$

$$\angle H(f) = - \arctan (f / f_B) \quad (8.5)$$

Figure (8.4 a & b) show the plots of the magnitude and phase-respectively- of the transfer function versus frequency (Allan R. Hambley 2008):

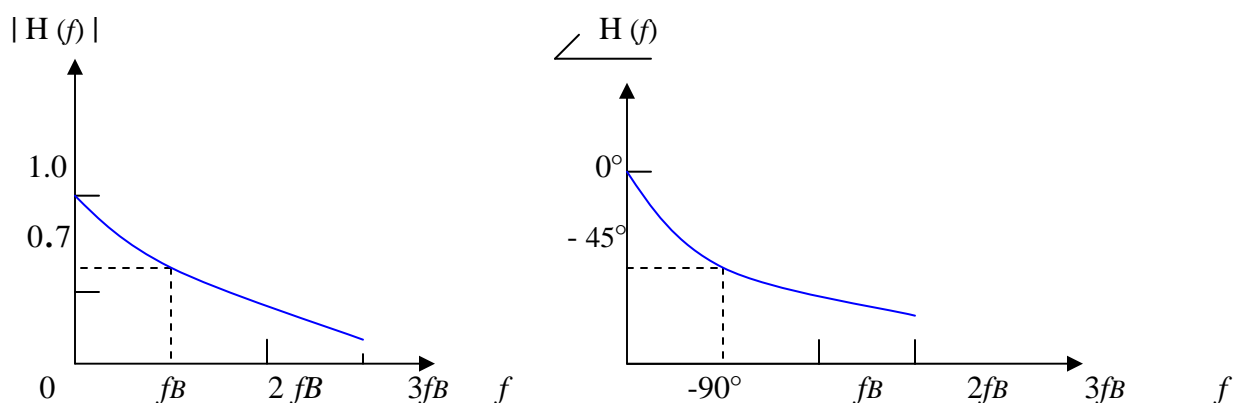


Figure 8.4: (a) The magnitude of the transfer function, (b) The phase of the transfer function (Image taken from (Allan R. Hambley 2008)).

It is seen from the plots that for low frequencies, the magnitude of the transfer function is approximately one and the phase is almost zero. This means low frequency components pass the filter to the output with out a considerable change. While for high frequency components in

contrast, the magnitude of the transfer function approaches zero and the phase approaches (-90). Thus, high frequency components have amplitude that is much smaller than the input amplitude and they are phase shifted as well.

The circuit was connected to the Arduino as shown in figure 8.5. A light sensor (light to frequency converter LTFC) **TSL235R** was placed on the Arduino and connected as shown in figure 8.5. The **TSL235R** combines a silicon photodiode and a current to frequency converter on a single integrated circuit. The device can be supplied with a voltage ranges between 2.7- 5.5 V, and responds to the light over the wavelength range (320- 1050 nm). Its output is a square wave with frequency proportional to the light intensity incident on the photodiode (www.sparkfun.com/products/9768). By connecting the Arduino to a computer and downloading the code - which is constructed to make the Arduino sense the light and map it to a voltage via appropriate functions – the desired range ($V_{out} = 0 - 10$ volts) was obtained and demonstrated on an Oscilloscope. The following section details the circuit performance.

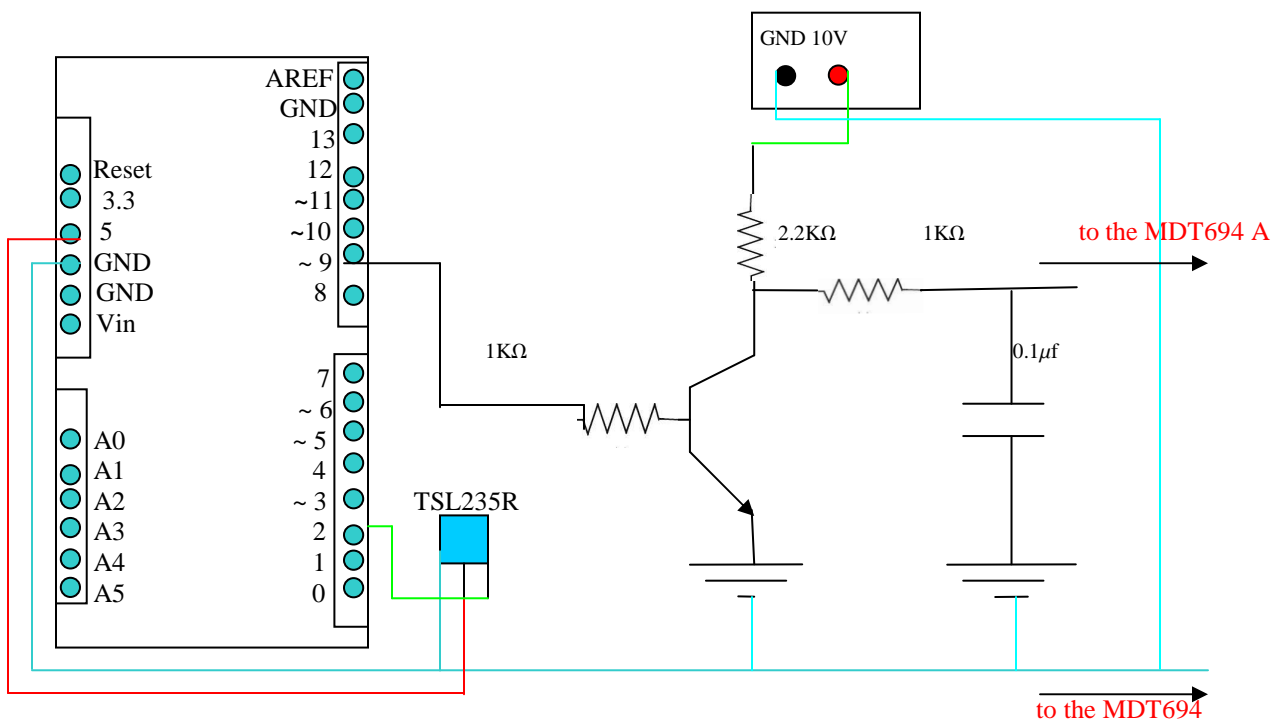


Figure 8.5: Piezo controlling circuit.

8.2 Circuit Performance:

Before connecting the circuit in figure 8.5 to the piezo controller, the output voltage was demonstrated on an oscilloscope to insure accessing the whole range (0-10 V). Explaining the circuit performance can be achieved via going through the **C code** – that was downloaded to the Arduino via the USB connection - as follows:

The first segment of the code declares the variables used in the code (either as integer or long which take different range of numerical values (see www.arduino.cc/en/reference/HomePage for details on data type)):

`int count2=0;` the value coming from the sensor.

`int Val;` the mapped value.

`volatile long count = 0;` variable to be updated by the interrupt.

`int threshold = 5000;` the maximum value from the photodiode (it changes depending on the maximum light intensity on the sensor).

`int transistorpin =9;` select the pin for transistor.

The second part of the code is the `setup ()` method which runs once (when the sketch starts):

`void setup ()`

`{ Serial.begin (9600);` turn on serial communication.

`attachInterrupt (0, MyIRQ, RISING);` enable interrupt 0 (pin2) –which is connected to the photodiode- to jump to the IRQ function (at the end of the sketch) on a Rising edge.

`pinMode (transistorpin, OUTPUT);` set the transistorpin as an output.

`TCCR1B=TCCR1B&0b11111000|0x01;` change the default frequency of pin 9.

`}`

This program watches pin 2 for a rising edge (could be change to falling) provided by the photodiode. This means watching for a voltage change going from logic low (0) to logic high (5), which will happen because of changing the light intensity on the photodiode. When this happens, the function `MyIRQ` is called and the code within this function is executed (as explained in chapter 7) and the variable `count` is incremented. The program then returned to where it was in the loop.

The loop () method runs continuously:

```
void loop()
{
  count2=int(count); convert count from long to integer.
  Val=map(count2,0,threshold,0,255); Val will range from 0-255.
  analogWrite(transistorpin,Val); put the mapped Val as an output on the transistor pin (pin 9).
  Serial.println(Val); print Val on the serial monitor.
  count=0; the updated variable.
  delay(100); wait 100 ms.
  return; return to the beginning of the loop.
}
```

The map function in this loop will scale the value from the sensor (count2) to the desired range (0-255) which corresponds to voltage ranges from 0 to 5 volts. This mapped Val will be put on pin 9 (that was set as an output and connected to the transistor). As a result the input voltage to the interface will range from 0-5 V and will be returned as an output voltage ranges from 0-10 V (as explained earlier).

The last part of the code is called interrupt service routine for interrupt 0. The code within this part works as explained in the setup method.

```
void MyIRQ()
{
  count++; increase the count variable.
  return; return to where it was in the program.
}
```

Figure 8.6 below shows V_{out} versus time as demonstrated on the oscilloscope before connecting the circuit to the piezo, where we see the linearity of V_{out} to insure accessing the desired range (0-10 V). Figures 8.7a & b show V_{out} versus time with out and with the filter respectively, which illustrates the effect of the filter as explained previously.



Figure 8.6: V_{out} versus time as obtained from the oscilloscope.

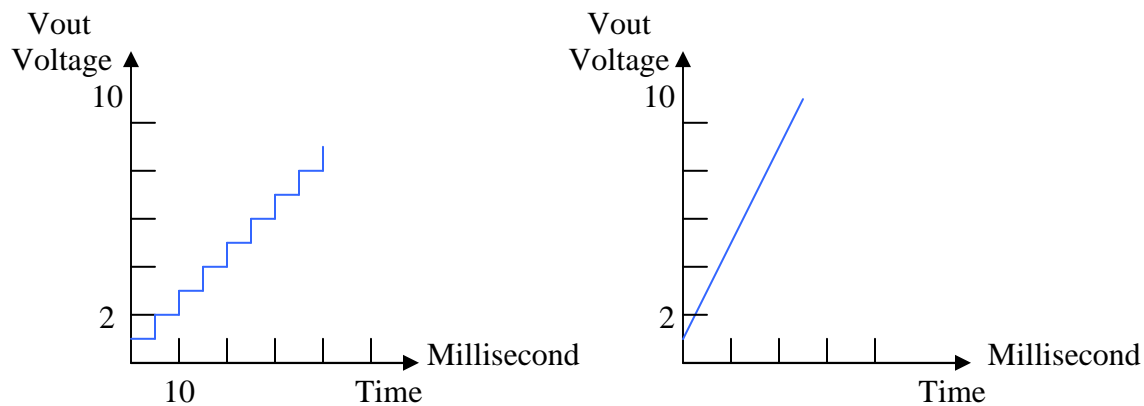


Figure 8.7: The picture illustrates (V_{out}) versus time: (a) With out the filter, (b) with the filter.

8.3 Conclusion and application to the field of microscopy:

Incorporating the circuit settings in figure 8.5 into a remote focusing system as shown in figure 8.8 will enable controlling the piezo via a signal that can be updated through changing the light intensity of the laser beam. As stated previously, the main goal of this work is keeping a stable focal plane of a system under investigation. This can be explained as follows:

The moving animal (or system) under investigation is represented by a circle in figure 8.8. When the system moves the light intensity of the laser beam will change (decrease or increase

based on the movement away or toward the objective lens). This change will be detected by the photodiode and V_{out} will decrease or increase accordingly (as explained in the previous section). Since the piezo is derived via this voltage (V_{out}) it is going to move away from or toward to (L2) changing the axial position of (M1) hence, changing the position of the focal plane accordingly (i.e. refocusing the microscope) (see chapter 6).

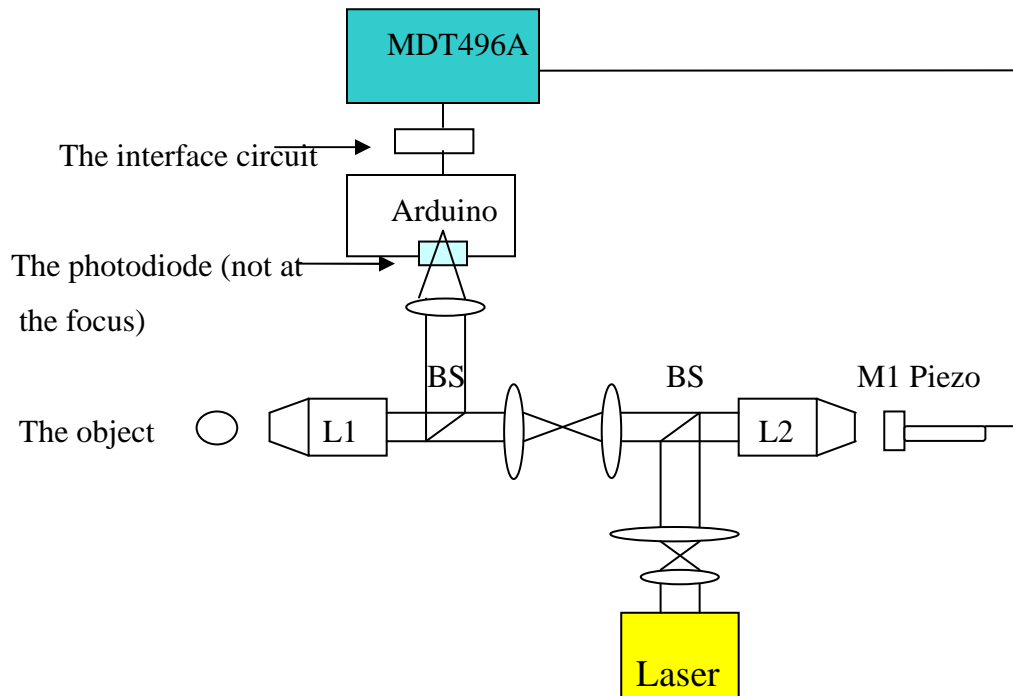


Figure 8.8: Schematic diagram of the remote focusing microscope linked to the piezo controlling circuit.

8.4 Review of the Achieved work:

The piezo used in this work has an axial range of (0-100 μ m). Connecting to the circuit the piezo was derived successfully in the lab by changing the light intensity of the laser beam incident on the photodiode. The response time to the change of the position of the focal plane is on the order of milliseconds. This means the desired focal plane is locked (in a period of time sufficient to biological systems) and the imaged information will not be lost. It is worth to note that the position of the photodiode should not be at the focus of the laser beam to sense the change in the light intensity (this is illustrated in figure 8.8).

REFERENCES CITED

- Adams, W.K., Perkins, K.K., Podolefsky, N. S., Dubson, M., Finkelstein, N.D., and Wie-
Man, C.E., 2006, New instrument for measuring student beliefs about physics and lea-
rning physics: The Colorado learning attitudes about science survey, *Phys. Rev.ST PE-
R. 2*: 10-101.
- Allan, R. H., 2008, *Electrical Engineering: Principles and applications*, 4th edn. *Person
Education Inc. New Jersey*.
- Andrea, D., 1988, Knowledge in pieces in *Constructivism in the computer Age* (ed. By: G.
Forman and P. Pufall). *Lawrence Erlbaum Associates, Hillsdale, New York* 49-70.
- Backskai, B.J., Kajdasz, S.T., Christie, R.H., Carter, C., Games, D., Seubert, P., Schenk,
D., and Hyman, B.T., 2001, Imaging of amyloid-beta deposits in brains of living mi-
Ce permits direct observations of clearance of plaques with immunotherapy, *Nat. M-
ed . 7*: 369-372.
- Botcherby, E., Juškaitis, R., Booth, M., and Wilson, T., 2007, Aberration-free optical re-
focusing in high numerical aperture microscopy, *Opt. Lett. 32*: 2007-2009
- Botcherby, E., Juškaitis, R., Booth, M., and Wilson, T., 2008 a, An optical technique for remo-
t focusing in microscopy, *Opt. Com. 281*: 880-887.
- Botcherby, E. , Juškaitis, R., Booth, M., and Wilson, T., 2008 b, Real-time extended depth of
field microscopy, *Optics Express*, 16.
- Brown, D.E., and David, H., 2008, Conceptual change in physics in *International Hndbo-
ok of Research on Conceptual Change* (ed. By S. Vosniadou), *New York* 127-154.
- Brown, E.B., Campbell, R.B., Tsuzuki, Y., Xu,L., Carmeliet, P., Fukumura, D., and jain, R.K.,
2001, in vivo measurement of gene expression, angiogenesis and physiological function in
tumors using multiphoton laser scanning microscopy, *Nat. Med. 7*:866-870.
- Callamaras, N., and Parker, I., 1999, Construction of a confocal microscope for real-time
x-y and x-z imaging, *Cell Calcium 26*: 271-279.
- Chabay, R., and Sherwood, B., 1997, Qualitative understanding and retention, *AAPT An-
nouncer 27*, 96.
- Chaigeneau, E., Oheim, M., Audinat, E., and Charpak, S., 2003, Two-photon imaging of capllar-
blood flow in olfactory bulb glomeruli, *Proc. Natl. Acad. Sci. USA 100* 13081- 3086.
- David, H., 1995, Epistemological considerations in teaching introductory physics, *Sci. Ed.*
79: 393-413.

- David, H., Malcolm, W., and Gregg, S., 1992, Force concept inventory, *Phys. Teach.* 30: 141-158.
- David, S., and Ronald, T., 1997, Using interactive lecture demonstrations to create an active learning environment, *Phys. Teach.* 35: 340-347.
- David, T., Lillian, M., 1980, Investigation of student understanding of the concept of velocity in one dimension, *Am. J. Phys.* 48:1020-1028.
- Denk, W., and Detwiler, P.B., 1999, Optical recording of light-evoked calcium signals in the functionally intact retina, *Proc. Natl. Acad. Sci. USA* 96: 7035-7040.
- Denk, W., Piston, D.w. and Webb, W.W., 2006, Two-photon molecular excitation in laser scanning microscopy, *Handbook of Biological Confocal Microscopy* 3rd edn (ed. By J.B Pawley), *Springer Science & Business Media, LLC, New York* 535-549.
- Denk, W., Strickler, J.H., and Webb, W.W., 1990, Two-photon laser scanning fluorescence microscopy, *Science* 248:73-76.
- Denk, W., Sugimori, M., and Llinas, R., 1995, Two types of calcium response limited to single spines in cerebellar Purkinje cells, *proc. Natl. Acad. Sci. USA* 92: 8279-8282.
- Ding, L., Chabay, R., Sherwood, B., and Beichner, R., 2006, Evaluating an Electricity and magnetism assessment tool: Brief electricity and magnetism assessment, *Phys. Rev. ST Phys. Educ. Res.* 2: 1-7.
- Edward, R., 2004, Theoretical frame work for physics education research: Modeling student thinking in *Proceedings of the International School of Physics, Enrico Fermi Course CLVI* (ed. By Edward R. and M. Vicentini), *IOS Press, Amsterdam*.
- Edward, R., Jeffrey, S., and Richard, S., 1998, Student expectations in introductory physics, *Am. J. Phys.* 66: 212-224.
- Egner, E., and Hell, W., 2006, *Handbook of Biological Confocal Microscopy*. 3rd edn, *Springer Science & Business Media, LLC, New York*.
- Engert, F., and Bonhoeffer, T., 1999, Dendritic changes associated with hippocampal long-term synaptic plasticity, *Nature* 399: 66-70.
- Finkelstein, N.M., Adams, W.K., Keller, C.J., Kohl, P.B., Perkins, K.K., Podolefsky, N.S., Reid, s., and LeMaster, R., 2005, When learning about the real world is better done virtually: A study of substituting computer simulations for laboratory equipment, *phys. Rev. ST PER* 1: 10-103.

- Fork, R.L., Martinez, O.E., and Gordon, J.P., 1984, Negative dispersion using pairs of prisms, *Opt. Lett.* 9: 150-152.
- Grutzendler, J., Kasthuri, N., and Gan, W.B., 2002, Long-term dendritic spine stability in the adult cortex, *Nature* 420 : 812-816.
- Heller, P., Foster, T., Heller, K., 1997, Cooperative group problem solving laboratories for introductory courses in *AIP Conference proceeding No. 399 The Changing role in Physics Department in Modern Universities: Proceeding of the International Conference on Undergraduate Physics Education*(ed. By E.F. Redish and J.S. Rigden), AIP press, New York 913-934.
- Hopt, A., and Neher, E., 2001, Highly nonlinear photodamage in two-photon fluorescence microscopy, *Biophys. J.* 80: 2029-2036.
- House, J.D., 1995, Student motivation, Previous instructional experience, and Prior achievement as predictors of performance in college mathematics, *International Journal of Instructional Media*, 22: 157-168.
- Huang, H., Vogel, S.S., Liu, N., Melton, D.A., and Lin, S., 2001, Analysis of pancreatic development in living transgenic zebrafish embryos, *Mol. Cell Endocrinal.* 177: 117- 124.
- Ibrahim, H., and David, H., 1985, The initial knowledge state of college physics students, *Am. J. Phys.* 53: 1043-1055.
- Ibrahim, H., Richard, H., and Eugene, M., 1995, Force concept inventory.
- Jane, C., 2007, Clickers in the large classroom: Current research and best-practice tips, *Life Sciences Education*, 6: 9-20.
- Jeffery, S., 1998, Beyond problem solving: Evaluating introductory physics courses through the hidden curriculum, PhD dissertation , *University of Maryland*.
- Karen, C., Jeffrey, M., Ronald, T., and Dennis, K., 1999, Evaluating innovation in studio Physics, *Am. J. Phys.* 67: S38-S44.
- Karen, W., 2001, Understanding, communication anxiety, and gender in physics: Taking the fear out of physics learning, *J. Coll. Sci. Teach.* 30: 232-237.
- Keller, U., 1994, Ultrafast all-solid-state laser technology, *Appl. Phys. B lasers opt.* 58: 347-363.
- Leonardo, H., Eric, B., Thomas, F., and Kathleen, H., 2004, Resource letter RPS-1: Research in problem solving, *Am. J. Phys.* 72: 1147-1156.

- Lillian, M., and Edward, R., 1999, Resource letter: PER-1: Physics education research, *Am. J. Phys.* 67: 755-767.
- Lillian, M., Peter, S., and Mark R., 1996, *Physics by Inquiry, Vol. 1, John Wiley and Sons, New York.*
- Mainen, Z.F., Malinow, R., and Svoboda, K., 1999b, Synaptic calcium transient in single spines indicate that NMDA receptors are not saturated, *Nature* 399: 151-155.
- Maloney, D., O’Kuma, T., Hieggelke, C., and Van, H., 2001, Surveying students’ conceptual knowledge of electricity and magnetism, *Am. J. Phys.* 69.
- Michelene, C., Paul, F., and Rober, G., 1981, Categorization and representation of experts and novices, *Cog. Sci.* 5: 121-152 .
- Neil, M.A., Juskaitis, R., Booth, M.J., Wilson, T., Tanka, T., Kawata, S., 2000, Adaptive Aberration correction in a two-photon microscope, *J. Microsc.* 200: 105-108.
- Neil, W., 1995, *Introductory statistics* , 4rd edn., *Addison Wesley.*
- Oku, H., Hashimoto, K., and Ishikawa, M., 2004, Variable-focus lens with 1-KHz band width *Opt. Express* 12: 21-38.
- Patricia, H., and Mark, H., Teaching problem solving through cooperative grouping. Part-2: Designing problems and structuring groups, *Am. J. Phys.* 60: 637-644.
- Patricia, H., Ronald, K., and Scott, A., 1992, Teaching problem solving through cooperative grouping . Part 1: Group versus individual problem solving, *Am.J. Phys.* 60: 627-636.
- Patterson, G.H., and Piston, D.W., 2000, Photobleaching in two-photon excitation microscopy, *Biophys. J.* 78: 2159-2162.
- Periasamy, A., Skoglund, P., Noakes, C., and Keller, R., 1999, An evaluation of two – photon excitation versus confocal and digital deconvolution fluorescence microscopy imaging in *Xenopus* morphogenesis, *Microsc. Res. Technol.* 47:172-181.
- Qi, B., Phillip, A.H., Maggie, L.G., Victor, X.D., David, L.D., and Alex, I.V., 2004, Dynamic focus control in high-speed optical coherence tomography based on a micro-electromechanical mirror, *Opt. Comm.* 232:123-128.
- Randall, K., 2004, *Physics for scientists and Engineers, Pearson Education Inc., Addison Wesley.*

- Raymond, S., Robert, B., and John, J., 2000, *Physics for Scientists and Engineers*. 5th edn. Saunders College Publishing, Forth Worth.
- Richard, B., 1994, Testing student interpretation of kinematics graphs, *Am. J. Phys.* 62: 750-762.
- Richard, H., 1998, Interactive-engagement vs. traditional methods: A six thousands-student survey of mechanics test data for introductory physics courses, *Am. J. Phys.* 66: 64-74.
- Robert, B., 1996, The impact of video motion analysis on kinematics graph interpretation skills, *Am. J. Phys.* 64: 1272-1277.
- Robert, B., 2009, An introduction to physics education research, *North Carolina State University, North Carolina*.
- Robert, B., Richard, H., Lillian, M., Jose, M., Edward, R., Fredrick, R., and John, R., 1995, Support of physics education research as a subfield of physics: Proposal to the NSF physics division.
- Ronald, T., and David, S., 1998, Assessing student learning of Newton's laws: The force and motion and the evaluation of active learning laboratory and lecture curricula, *Am. J. Phys.* 66: 338-352.
- Sherman, L., Ye, J.Y., Albert, O., Norris, T. B., 2002, Adaptive correction of depth-induced aberrations in multiphoton scanning microscopy using a deformable mirror. *J. Microsc.* 206: 65-71.
- Spence, D.E., Kean, P.N., and Sibbett, W., 1991, 60-fsec pulse generation from a self-mode-locked Ti: sapphire laser, *opt. Lett.* 16: 42-44.
- Summers, R.G., Piston, D.W., Harris, K.M. and Morrill, J.B., 1996, The orientation of first cleavage in the sea urchin embryo, *Lytechinus variegatus*, does not specify the axes of bilateral symmetry, *Dev.Biol.* 175: 177-183.

APPENDIX A- THE TRANSLATED CONCEPTUA SURVEY OF PHYSIC (TCSP)

A1. Two metal balls have the same diameter, but one is twice as massive as the other. The balls are dropped from the roof of a single story building at the same instant. The time it takes the balls to reach the ground will be:

- (A) About half as long for the heavier ball as for the lighter one.
- (B) About half as long for the lighter ball as for the heavier one.
- (C) About the same for both balls.
- (D) Considerably less for the heavier ball, but not necessarily half as long.
- (E) Considerably less for the lighter ball, but not necessarily half as long.

A2. A large truck collides head-on with a small compact car. During the collision:

- (A) The truck exerts a greater amount of force on the car than the car exerts on the tuck.
- (B) The car exerts a greater amount of force on the truck than the truck exerts on the car.
- (C) Neither exerts a force on the other, the car smashed simply because it gets in the way of the truck .
- (D) The truck exerts a force on the car but the car dose not exert a force on the truck.
- (E) The truck exerts the same amount of force on the car as the car exerts on the truck .

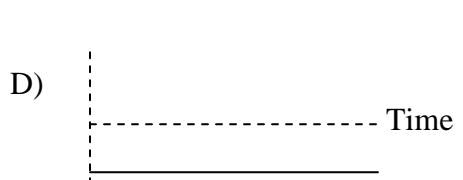
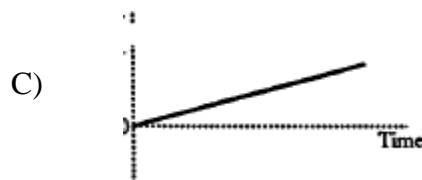
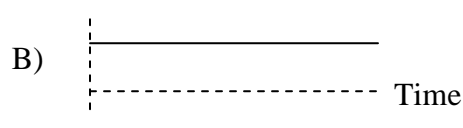
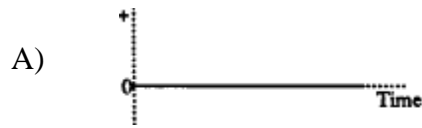
The graphs refer to a toy car which can move to the left or right along a horizontal line(the positive part of the distance axis).The positive direction is to the right. Different motions of the car are described in each statement below .Choose the letter (A to D)of the graph which corresponds to the car motion in each statement. You may use a choice more than once or not at all. If you think that none is correct, choose (E).

A3. The car moves toward the right and is speeding up at steady rate(constant acceleration). Find a graph showing velocity versus time?

A4. The car moves toward the left (toward the origin) at a constant velocity. Find a graph showing acceleration versus time?

A5. The car moves toward the right and is slowing down at steady rate (constant acceleration).Find a graph showing velocity versus time.

A6. The car moves toward the right and is speeding up at steady rate. Find a graph showing acceleration versus time.



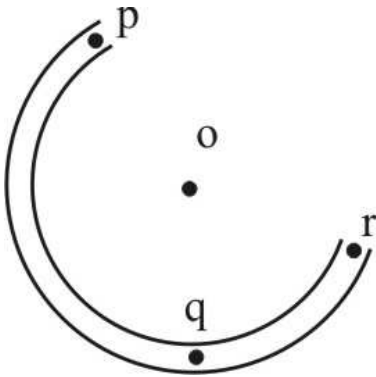
E) No graph is correct.

- A7. The accompanying figure shows a frictionless channel in the shape of a segment of circle with its center at the point marked "O". The channel has been anchored to a frictionless horizontal table top. In the figure, you are looking down at the table. Forces exerted by the air are negligible. We shoot a ball at high speed into the channel at "p" and the ball exits at "r".

Consider the following distinct forces:

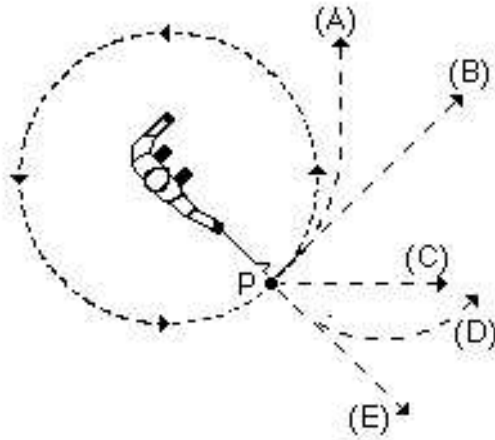
- I. A downward force of gravity.
- II. A force exerted by the channel pointing from q to o.
- III. A force in the direction of motion.
- IV. A force pointing from o to q.

Which of the above forces is (are) acting on the ball when it is within the frictionless channel at position "q"?

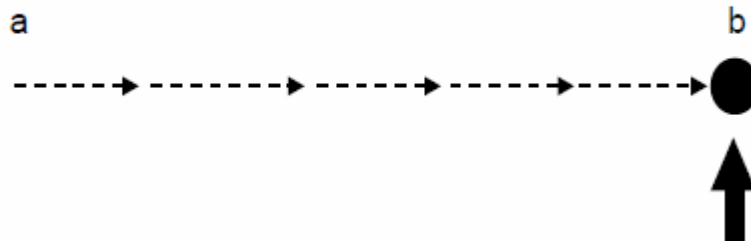


- (A) I only.
- (B) I and II.
- (C) I and III.
- (D) I, II, and III .
- (E) VI,,II,IV

- A8. A steel ball is attached to a string and is swung in a circular path in a horizontal plane as illustrated in the accompanying figure. At the point P indicated in the figure, the string suddenly breaks near the ball. If these events are observed from directly above as in the figure, which path would the ball most closely follow after the string breaks?



- A9. The next figure depicts a hockey puck sliding with constant speed v_o in a straight line from point "a" to point "b" on a frictionless horizontal surface. Forces exerted by the air are negligible. You are looking down on the puck. When the puck reaches point "b," it receives a swift horizontal kick in the direction of the heavy print arrow. Had the puck been at rest at point "b," then the kick would have set the puck in horizontal motion with a speed v_k in the direction of the kick.



- The speed of the puck just after it receives the kick is:
- (A) equal to the speed v_o it had before it received the kick.
 - (B) equal to the speed v_k resulting from the kick and independent of the speed v_o .
 - (C) equal to the sum of the speeds v_o and v_k .
 - (D) smaller than either of the speeds v_o or v_k .
 - (E) greater than either of the speeds v_o or v_k , but less than the sum of these two speeds.

A10. A boy throws a steel ball straight up. Consider the motion of the ball only after it has left the boy's hand but before it touches the ground, and assume that forces exerted by the air are negligible. For these conditions, the force(s) acting on the ball is (are):

- (A) a downward force of gravity along with a steadily decreasing upward force.
- (B) a steadily decreasing upward force from the moment it leaves the boy's hand until it reaches its highest point; on the way down there is a steadily increasing downward force of gravity as the object gets closer to the earth.
- (C) an almost constant downward force of gravity along with an upward force that steadily decreases until the ball reaches its highest point; on the way down there is only a constant downward force of gravity.
- (D) an almost constant downward force of gravity only.
- (E) none of the above. The ball falls back to ground because of its natural tendency to come to rest on the surface of the earth.

A11. A large truck breaks down out on the road and receives a push back into town by a small compact car as shown in the figure below:

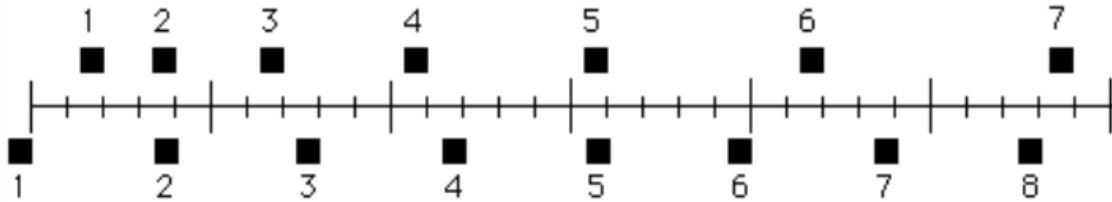


While the car, still pushing the truck, is speeding up to get up to cruising speed:

- (A) the amount of force with which the car pushes on the truck is equal to that with which the truck pushes back on the car.
- (B) the amount of force with which the car pushes on the truck is smaller than that with which the truck pushes back on the car.
- (C) the amount of force with which the car pushes on the truck is greater than that with which the truck pushes back on the car.
- (D) the car's engine is running so the car pushes against the truck, but the truck's engine is not running so the truck cannot push back against the car. The truck is pushed forward simply because it is in the way of the car.
- (E) neither the car nor the truck exert any force on the other. The truck is pushed forward simply because it is in the way of the car.

- A12. The positions of two blocks (A&B) at successive 0.20-second time intervals are represented by the numbered squares in the figure below. The blocks are moving toward the right.

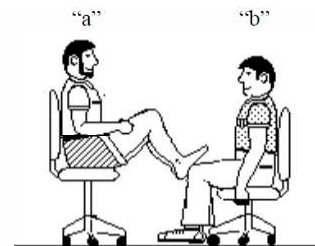
Block A



Block B

Do the blocks ever have the same speed?

- (A) No.
 (B) Yes, at instant 2.
 (C) Yes, at instant 5.
 (D) Yes, at instants 2 and 5.
 (E) Yes, at some time during the interval 3 to 4.
- A13. A woman exerts a constant horizontal force on a large box. As a result, the box moves across a horizontal floor at a constant speed v_0 . The constant horizontal force applied by the woman:
- (A) has the same magnitude as the weight of the box.
 (B) is greater than the weight of the box.
 (C) has the same magnitude as the total force which resists the motion of the box.
 (D) is greater than the total force which resists the motion of the box.
 (E) is greater than either the weight of the box or the total force which resists its motion
- A14. In the figure at right, student "a" has a mass of 95 kg and student "b" has a mass of 77 kg. They sit in identical office chairs facing each other. Student "a" places his bare feet on the knees of student "b", as shown. Student "a" then suddenly pushes outward with his feet, causing both chairs to move. During the push and while the students are still touching one another:



- (A) neither student exerts a force on the other.
 (B) student "a" exerts a force on student "b", but "b" does not exert any force on "a".
 (C) each student exerts a force on the other, but "b" exerts the larger force.
 (D) each student exerts a force on the other, but "a" exerts the larger force.
 (E) each student exerts the same amount of force on the other.

- A15. Despite a very strong wind, a tennis player manages to hit a tennis ball with her racquet so that the ball passes over the net and lands in her opponent's court.

Consider the following forces:

1. A downward force of gravity.
2. A force by the "hit".
3. A force exerted by the air.

Which of the above forces is (are) acting on the tennis ball after it has left contact with the racquet and before it touches the ground?

- (A) 1 only.
(B) 1 and 2.
(C) 1 and 3.
(D) 2 and 3.
(E) 1, 2, and 3.

Two small objects each with a net charge of $+Q$ exert a force of magnitude F on each other:

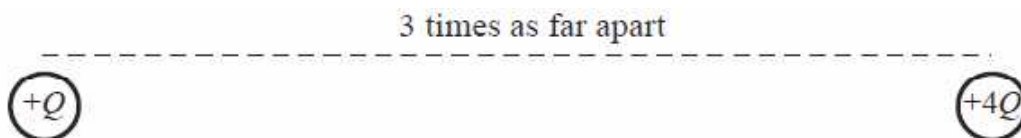


We replace one of the objects with another whose net charge is $+4Q$:



- A16. The original magnitude of the force on the $+Q$ charge was F . What is the magnitude of the force on the $+Q$ charge now?
- (A) $16F$
(B) $4F$
(C) F
(D) $F/4$
(E) None of the above

Next we move the $+Q$ and $+4Q$ charges to be 3 times as far apart as they were:

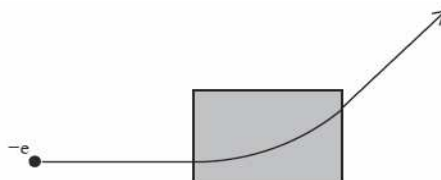


A17. Now what is the magnitude of the force on the $+4Q$ charge?

- (A) $F/9$
- (B) $F/3$
- (C) $4F/9$
- (D) $4F/3$
- (E) None of the above

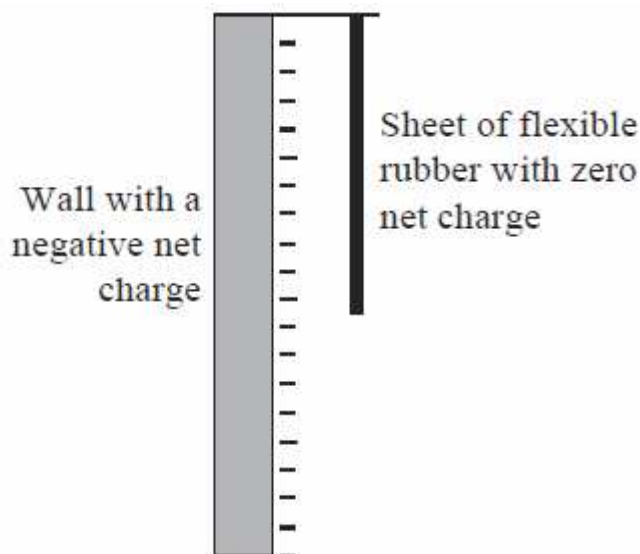
A18. A moving electron with charge $-e$ travels along the path shown, and passes through a region of electric field. There are no other charges present. The electric field is zero everywhere except in the gray region.

What is a possible direction (a - g) of the electric field in the region where the field is non- zero?



- A) Into the page
- B) Out of the page
- C) Up
- D) Down
- E) Along the path of the electron

A19. A non-conducting wall is given a net negative charge. Next, a sheet of very flexible rubber with zero net charge is suspended from the ceiling near the charged wall as shown below.



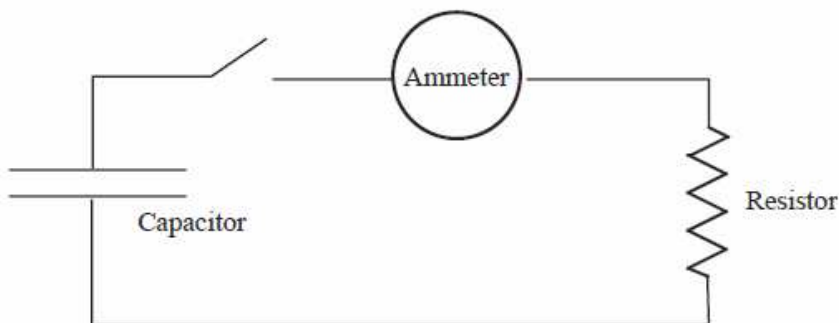
Initially, the rubber sheet will:

- (A) not be affected by the charges on the wall since rubber is an insulator.
- (B) not be affected by the charged wall because the rubber sheet has zero net charge.
- (C) bend away from the wall due to the electrical repulsion between the electrons in the rubber and the charges on the wall.
- (D) bend away from the wall due to the polarization of the rubber molecules by the charged wall.
- (E) bend toward the wall due to the polarization of the rubber molecules by the charged wall.

A20. In static equilibrium, the potential difference between two points inside a solid piece of metal

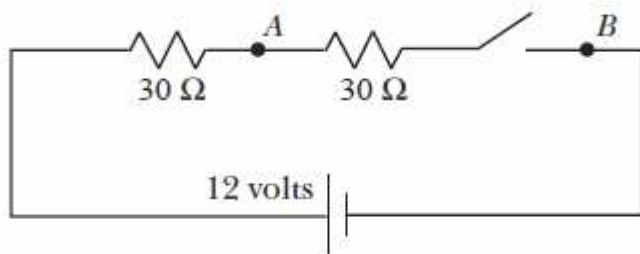
- (A) is zero because metals block electric interactions
- (B) is zero because the electric field is zero inside the metal
- (C) is non-zero if there are charges on the surface of the metal
- (D) is non-zero for reasons not given above.
- (E) is zero for reasons not given above.

A21. A capacitor is originally charged. How does the current I in the ammeter behave as a function of time after the switch is closed?

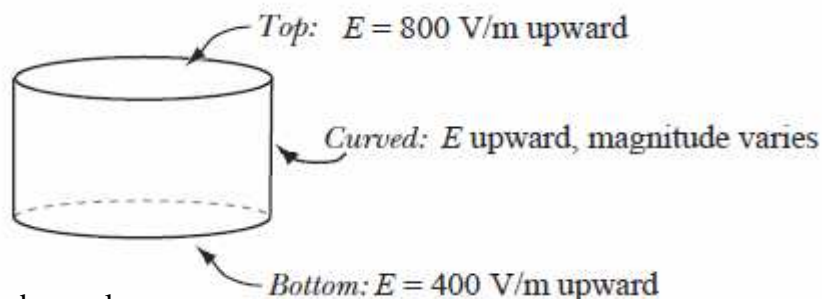


- (A) $I = 0$ always
- (B) $I = \text{constant} \neq 0$
- (C) I increases, then is constant.
- (D) I instantly jumps up, then slowly decreases.
- (E) None of the above

- A22. What is the magnitude of the potential difference between points A and B on the circuit shown below, while the switch is open?



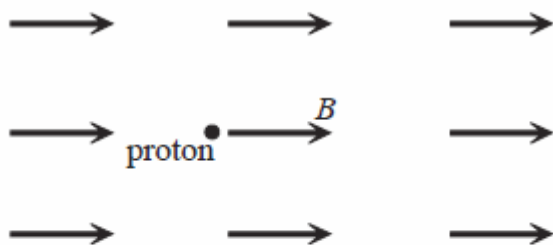
- (A) 0 volts
 (B) 3 volts
 (C) 6 volts
 (D) 12 volts
 (eE None of the above.
- A23. Here is a cylinder on whose surfaces there is an electric field whose direction is vertically upward, but whose magnitude varies as shown.



The cylinder encloses

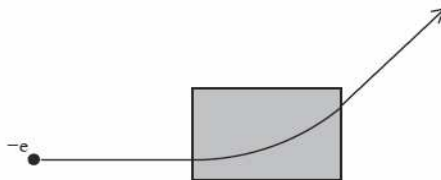
- (A) no net charge.
 (B) net positive charge.
 (C) net negative charge.
 (D) There is not enough information available to determine whether or not there is net charge inside the cylinder.

- A24. A proton is initially at rest in a region of uniform magnetic field (shown below). There are no other charges present.



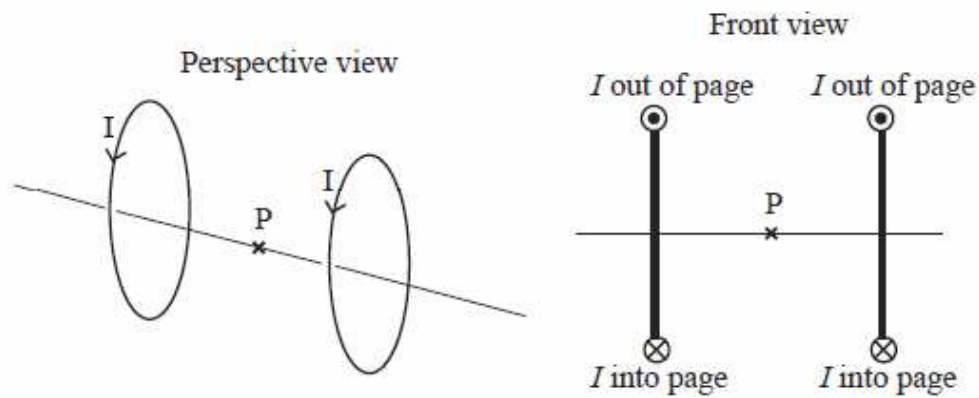
What is the direction (a-h) of the initial magnetic force on the proton?

- A) Up
 - B) Out of the page
 - C) Into the page
 - D) Zero magnitude
 - E) None of the above
- A25. A moving electron travels along the path shown, and passes through a region of magnetic field. There are no other charges present. The magnetic field is zero everywhere except in the gray region.



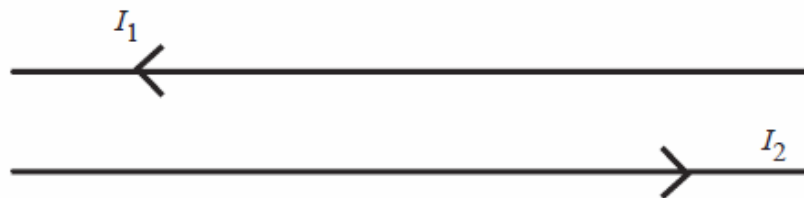
- A) Into the page
- B) Out of the page
- C) Up
- D) Down
- E) Along the path of the electron

- A26. Two identical circular loops of wire, perpendicular to the page, carry the same conventional current I :



What is a possible direction of the magnetic field due to the loops at location P, which is midway between the loop?

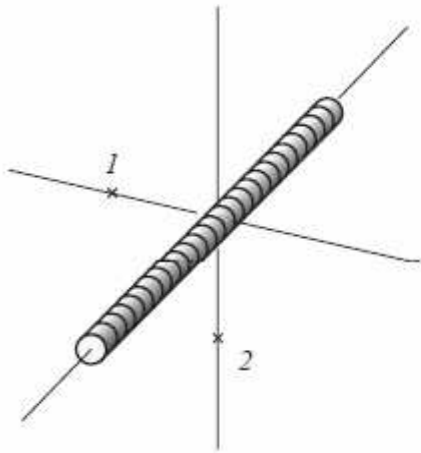
- A) Out of the page
 - B) Into the page
 - C) Left
 - D) Right
 - E) Zero magnitude
- A27. Two wires lie in the plane of the page. Wire 1 carries conventional current to the left, and wire 2 carries conventional current to the right:



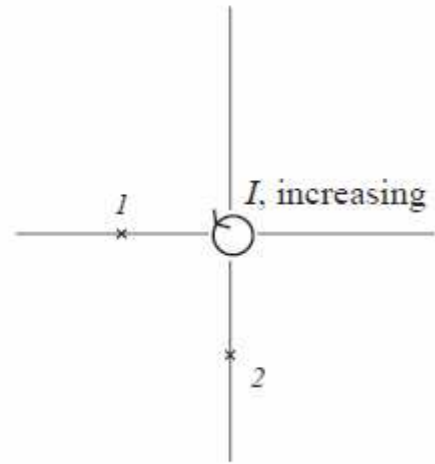
What is the direction of the magnetic force that wire 1 exerts on wire 2?

- A) Up
- B) Down
- C) Into the Page
- D) Out of the page
- E) Zero force

Here is a long solenoid (coils of wire along a long cylinder) and an end view of the solenoid.
 Conventional current runs counter-clockwise in the solenoid and is increasing with time.



Long solenoid, perspective view



Solenoid, end view

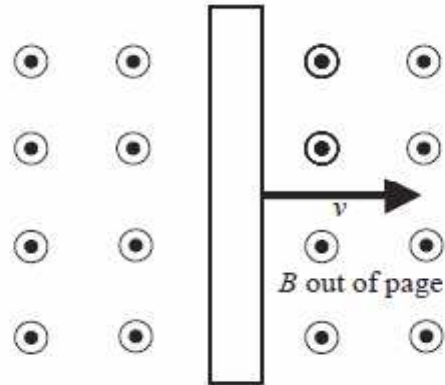
A28. What is the direction of the *electric* field at location 1 (marked with \times)?

- A) Left
- B) Right
- C) Down
- D) Zero field
- E) None of the above

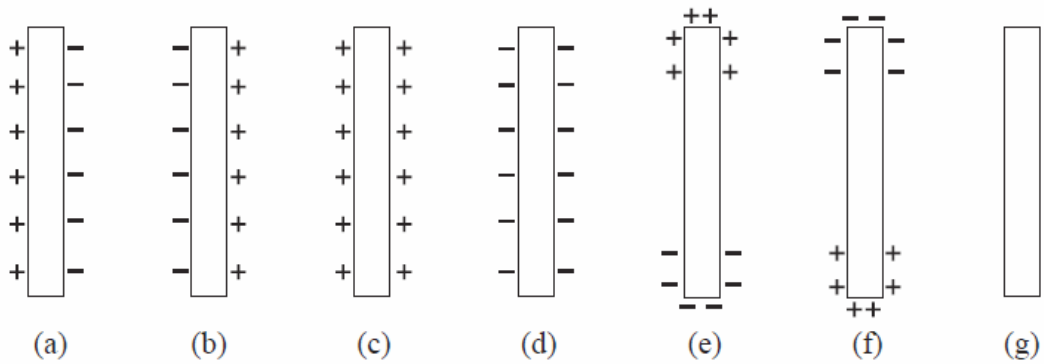
A29. What is the direction of the *electric* field at location 2 (marked with \times)?

- A) Up
- B) Down
- C) Left
- D) Zero field
- E) None of the above

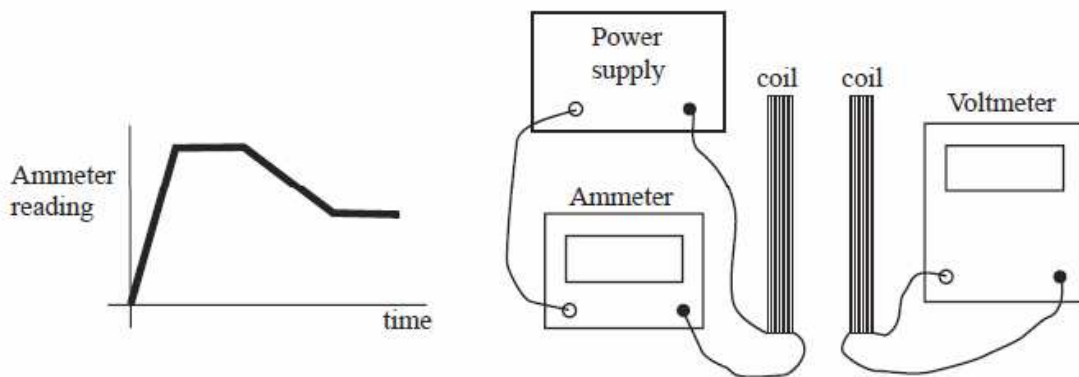
- A30. A neutral metal bar is moving at constant velocity to the right through a region where there is a uniform magnetic field pointing out of the page. The magnetic field is produced by some large coils which are not shown on the diagram.



Which of the following diagrams best describes the state of the metal bar?



- A31. A variable power supply is connected to a coil and an ammeter, and the time dependence of the ammeter reading is shown. A nearby coil is connected to a voltmeter.



Which of the following graphs correctly shows the time dependence of the voltmeter reading?

